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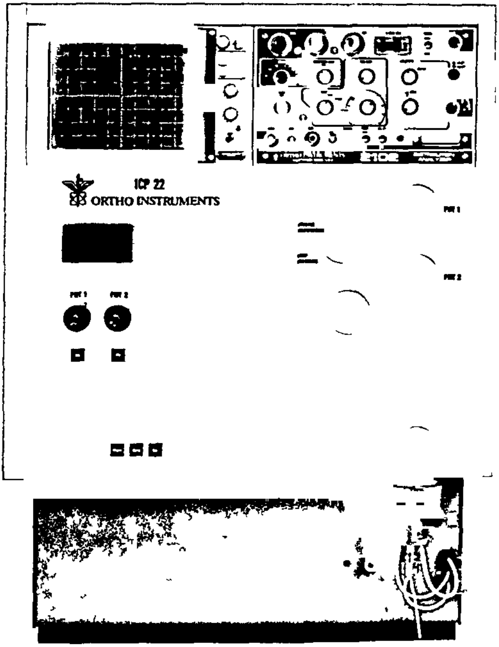

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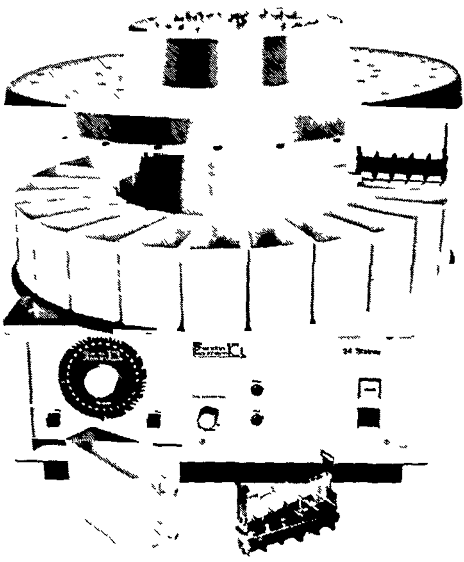

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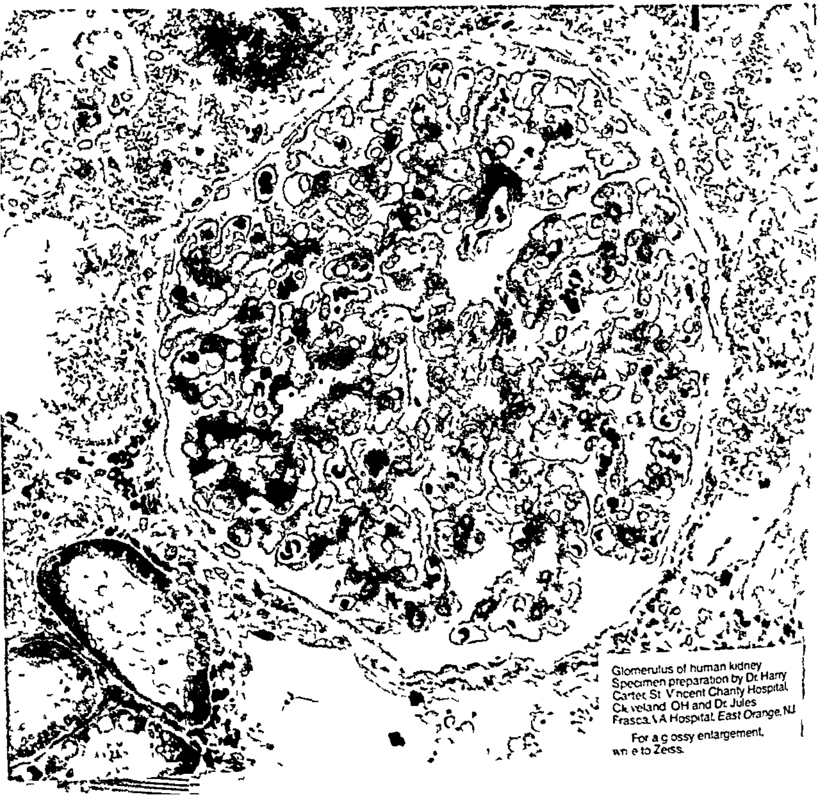

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ACTA CYTOLOGICA

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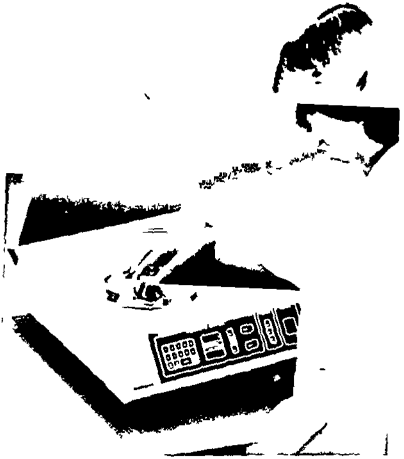

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Editorial

Thin Needle Aspiration Biopsy

The distinguished British medical publication *The Lancet* recently carried a thought provoking article on the subject of thin needle aspiration biopsy [1] The article explores the reasons why this method of diagnosis has become immensely popular in the Scandinavian countries notably in Sweden but has made so far limited inroads in the United States The author Dr Cecil Fox although neither a pathologist nor a cytopathologist has a great deal of interest in and knowledge of cells Because he has no vested interest in diagnostic cytology (except as an interested observer) his remarks warrant careful study

Several reasons are cited by Fox as a possible explanation for the disparity between Sweden and the United States (1) difficulties in obtaining properly trained personnel (2) fears of lawsuits (3) fears that the technique is a second class method of diagnosis and (4) fears of spread of tumor along the needle track Perhaps the most important point in Fox s argument is the fear of clinicians *and pathologists* alike of adopting an unproven subjective method of diagnosis that requires *significant* experience to achieve acceptable diagnostic accuracy Fox contrasts this distrust of aspiration biopsy with the rapid acceptance of new objective methods of diagnosis for example those based on measurable chemical parameters

In my judgment there is considerable truth in Cecil Fox s arguments although the matter is significantly more complex and has to do with *historical* developments in pathology in the United States and in Europe The recognition of the value of a tissue biopsy occurred in Europe roughly 100 years ago as a direct extension of the morphologic classification of disease by the successors of Rudolph Virchow With in the first two decades of this century the biopsy became accepted as a tool of accurate identification of disease throughout the world This method of diagnosis and the role of the pathologist in the identification of disease processes received an enthusiastic endorsement in the United States Consequently after the conclusion of World War II a major system of hospital accreditation was developed that required the availability of a skilled certified specialist in pathology in *every* hospital and determined that no human tissue may be removed without the benefit of a pathologic examination Thus in the U S A an excellent system of rapid and reliable tissue diagnosis available to all inpatients and outpatients has been in place for many years This is still not the case in many European countries even in Germany and Scandinavia where large hospitals may function *without* the services of a qualified pathologist As a consequence in many European countries biopsies often have to be forwarded for diagnosis to central pathology laboratories (usually medical schools) occasionally with very substantial delays in diagnosis It would be inconceivable in the U S A to have a delay of two to three weeks in the diagnosis of let us say a carcinoma of the breast because the central referral laboratory may be swamped with samples from peripheral hospitals or clinics

Another factor of importance may be the availability of skilled pathologists for diagnostic purposes The ratio of practicing diagnostically skilled pathologists per unit of population is difficult to estimate because no reliable figures are available While the number of skilled American pathologists grew rapidly after 1945 to the present level of approximately 10 000 (approximately one pathologist per 20 000 persons) the number of pathologists in Europe appears to be much smaller for the population perhaps one per 30 000 to 40 000 persons These tentative figures do not reflect the ratio of academic or research oriented pathologists to practicing pathologists which is impossible to estimate In general in Europe hospitals which are not affiliated with medical schools are not generously staffed by pathologists

In the U S A the pathologist is in general a well accepted member of the clinical team His or her

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diagnostic acumen and contributions are often sought and appreciated It is not uncommon in Europe to see diagnostic laboratories operated by clinical departments either because a pathologist is not available or because the pathologists diagnostic skills are judged inadequate for the clinical needs It is against this background that the acceptance of the aspiration biopsy must also be judged This method provided a much needed method of diagnosis in situations where a rapid and reliable diagnostic verdict was otherwise not obtainable

In the eyes of many pathologists and clinicians in the United States the efficacy and reliability of the aspiration method when compared with the histologic approach to diagnosis have not been adequately tested as yet except perhaps for the breast There is little doubt that in experienced hands the smear diagnosis of cancer is accurate and reliable,[4] particularly when confirmatory of an obvious clinical setting However the diagnostic difficulties inherent in the method have not received sufficient emphasis

I have been familiar with the aspiration technique for the past 27 years During this period I have witnessed quite a few diagnostic errors committed by experienced observers in the interpretation of aspirated material occasionally with calamitous effects for the patient The 100% accuracy cannot be achieved with any method of diagnosis but a truly comprehensive review of the difficulties in the interpretation of aspiration biopsy smears based on a major experience in a difficult clinical setting has not been published to date It is obvious that as the aspiration biopsy smear is applied to an ever widening number of organs and tissue targets the practitioners of the method must master the entire field of human pathology It is simply not enough to have a reasonable command of criteria separating benign from cancer cells because they may vary enormously from organ to organ from patient to patient and from disease to disease The same diagnostic parameters when applied to different organs will not work and may in fact result in diagnostic disasters Most contemporary surgeons or internists are sophisticated enough to know that within the same organ there may occur malignant processes of unequal value of different prognosis and requiring different approaches to treatment Furthermore benign processes imitating cancer are common enough to frighten even the most intrepid and experienced diagnostician

A further difficulty has to do with the actual performance of the aspiration procedure The Swedish experience suggests that the best results are obtained when the cytologist is also the person performing the aspiration This may well be so but again is contrary to the American experience and tradition With the exception of bone marrow aspirates (and this usually only in the absence of a clinical hematologist) the American pathologist is generally not trained to perform clinical procedures and often is too busy to devote much time to such activities although there are some exceptions[2] If the American pathologist were to enter the field of aspiration biopsy as the actual performer of the clinical procedure he or she would also enter into a major conflict with the clinicians who are generally reluctant to give up this privilege Thus the Swedish experience with aspiration biopsies has produced a professional hybrid a clinical cytologist who evaluates the patients and performs and evaluates the aspiration biopsy but who is often not a pathologist and thus must rely on others for confirmation of the diagnosis

Nonetheless it is my deep conviction that the thin needle aspiration biopsy is a procedure whose time has come The aspiration can often be performed as an office procedure and thus saves time and money The diagnoses in competent hands are usually quite accurate and the difficulties of interpretation can be mastered with experience Samples can be obtained from areas of the body not previously accessible to morphologic diagnosis without major often debilitating and sometimes unnecessary exploratory surgery[3]

The great danger of the method lies in erroneous interpretation of the sample by inexperienced observers without sufficient background in tissue pathology and without sufficient cognizance of the many diagnostic pitfalls The national and international Societies and qualifying specialty Boards interested in ensuring very high standards of performance in aspiration biopsy would do well to establish the necessary requirements of training and experience that would secure competent performance of this very important procedure for the maximal benefit of the patient There is little doubt that with the passage of time the thin needle aspiration biopsy will in part replace frozen sections and tissue biopsies particularly for the diagnosis of cancer However wisdom and experience to date would suggest that a slow cautious approach coupled with appropriate control studies would be most beneficial to pathologists and patients alike

Leopold G Koss M D F I A C

References

1 Fox CH Innovation in medical diagnosis The Scandinavian curiosity Lancet 1 1387 1388 1979

2 Frable WJ Thin-needle aspiration biopsy A personal experience with 469 cases Am J Clin Pathol 65 168 18 1976

3 Koss LG Thin needle aspiration biopsy of endocrine organs Ann Clin Lab Sci 9 3 6-33 1979

4 Zajicek J The aspiration biopsy smear *In* Diagnostic Cytology and its Histopathologic Bases Edited by LG Koss Third edition Philadelphia JB Lippincott 1979 pp 1001 1104

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Cytologic Diagnosis of Intraventricular Hemorrhage in a Neonate

Peter G Bernad, M D
Priscilla D Taft, M D

A neonate with autoimmune thrombocytopenia presented with megacephaly and hydrocephalus The cause remained obscure until hemosiderin laden macrophages were found in the ventricular cerebrospinal fluid (CSF) indicating prenatal intracranial hemorrhage

The literature on the diagnosis of intraventricular hemorrhage by the cytologic examination of CSF is reviewed and discussed The cytologic evaluation of CSF may be helpful in cases of central nervous system dysfunction when the cause is obscure

The cytologic analysis of cerebrospinal fluid (CSF) allows the diagnosis of certain neoplasms and infections of the central nervous system As cytologic techniques are applied more widely diagnoses can also be made of central nervous system lesions of obscure origin[1-3] We wish to record the cytologic diagnosis of cerebral intraventricular hemorrhage in a newborn infant who also had hydrocephalus and megacephaly

Case Report

A 12-hour-old female infant was transferred to the Massachusetts General Hospital (MGH) because of an enlarging head and thrombocytopenia She was born at 38 weeks gestation of a healthy mother (gravida 3 paragravida 3) There were no complications except for maternal exposure to varicella in the second trimester of the pregnancy

The baby was delivered by a vertex presentation under local block anesthesia She weighed 3 35 kg with Apgar scores of ten at one and five minutes No abnormalities were noted except for an enlarged head (39 cm circumference), which was over the 97th percentile Later in the same day the child was noted to have 7 000 platelets per milliliter and petechiae over the back front and limbs and was transferred to the MGH

On admission the infant was megacephalic and had a markedly increased cranial circumference widened cranial sutures a full frontal fontanelle and numerous petechiae over the trunk, limbs and palate Retinal hemorrhages and hepatosplenomegaly were noted The neurologic examination suggested hydrocephalus The platelet count was 3 000 per milliliter The electroencephalogram (EEG) was mildly abnormal with posterior sharp waves A computerized axial tomographic (CAT) scan of the head demonstrated cystic lesions in the cerebrum and hydrocephalus and questionable intracerebral calcification (Figure 1) An infectious condition such as toxoplasmosis was considered Treatment consisted of platelet transfusion steroids and exchange transfusion Because of the hydrocephalus a right cerebral ventricular peritoneal shunt was performed Since chronic intraventricular hemorrhage *in utero* was also considered to be a possibility the ventricular CSF was examined cytologically with a Millipore filter preparation and smears of the deposit obtained by centrifugation These preparations were stained with Papanicolaou stain and by Prussian blue stain for iron They demonstrated numerous hemosiderin laden

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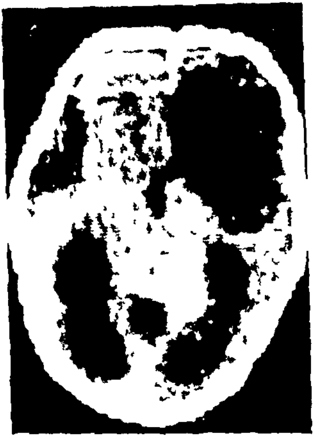

Figure 1
CAT scan without contrast at the level of the lateral ventricles, showing large cystic lesions, hydrocephalus and an area of increased attenuation values on the right, suggesting calcification.

macrophages (Figures 2 and 3). All the cultures and serologic examinations for the presence of microorganisms were negative.

After the shunt was inserted, the circumference of the head decreased, approaching normal. A follow-up EEG was normal. A repeat CAT scan showed smaller cerebral ventricles. At follow-up one month later the child was thriving, with a stable platelet count of 150,000 per milliliter.

Discussion

The cytologic examination of cerebral ventricular fluid may be helpful in patients who are suspected of having primary or metastatic neoplasms in the brain, who are undergoing craniotomy or who have obscure diagnostic problems. An examination for evidence of previous hemorrhage is rare.[1] This technique seems useful in analyzing cases of obscure hydrocephalus, of central nervous system lesions associated with platelet or coagulation defects and of suspected intracranial hemorrhage. Cytologic analysis might also assist in determining the time of the bleed.

When blood enters the CSF, a leptomeningeal cell reaction takes place[3] with the appearance of granulocytes. After about four hours the first signs of phagocytosis appear. Monocytes, the first to undergo cytoplasmic activation, initiate phagocytosis but are not effective in eliminating the numerous red blood cells (RBCs). Active proliferation of leptomeningeal tissues occurs at about 12 to 18 hours. Many macrophages appear, and RBCs in contact with them are engulfed within a few hours. Enzymatic destruction of the hemoglobin occurs first, with RBCs appearing as empty cytoplasmic vacuoles in the macrophages. Destruction of the more resistant stroma of RBCs takes longer. At about four days the first hemosiderin granules are seen in the cytoplasm of phagocytes,[4,5] often referred to as "hemosiderophages." Hemosiderin usually appears in the cytoplasm as large, coarse, dark brown to gray-black granules or finely distributed minute granules.[4] Hematoidin is deposited in the cytoplasm somewhat later, usually as brownish yellow granules.

The life span of phagocytes is relatively long, and they can still be found in the subarachnoid space more than six months after the initial hemorrhage.[2] A long life span can be simulated when aging macrophages and their contents are in turn phagocytized by younger cells. Macrophages can provide evidence not only of a single hemorrhage but also of repeated hemorrhages. In the case of repeated hemorrhage, recently ingested RBCs, bleached RBCs, hemosiderin and hematoidin are simultaneously present in the cytoplasm.

The Prussian blue stain is specific for iron in the ferric form. It provides conclusive evidence of the presence of hemosiderin and distinguishes it from other types of brown granules in the cytoplasm (Figure 3).

In the newborn infant reported above, suspected intraventricular cerebral hemorrhage was confirmed by cytologic examination of ventricular CSF. In addition, since the macrophages contained granules rather than RBCs, the hemorrhage could be dated as having occurred *in utero*. The Prussian blue stain demonstrated conclusively that the granules were derived from the breakdown of hemoglobin rather than from some other source since they contained iron. Cytologic examination and specific staining may establish such a diagnosis in other obscure cases.

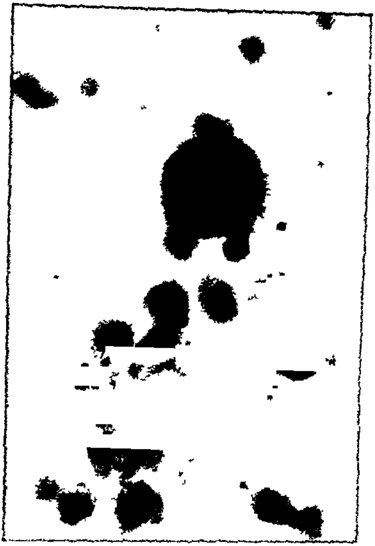

Figure 2
Ventricular CSF showing macrophages with brownish granules of hemosiderin (Papanicolaou stain × 570)

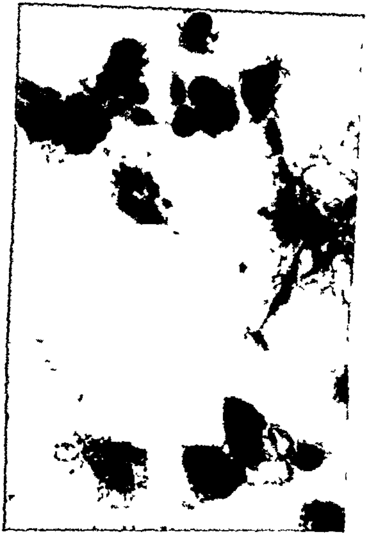

Figure 3
*Ventricular CSF with Prussian blue reaction confirming the pres*ence of iron (stained blue) in the granules (× 570)

References

1 Kolmel HW Atlas of Cerebrospinal Fluid Cells New York *Springer Verlag 1976 pp 30-33*

2 Muller R Sornas R CSF cytology in cerebrovascular disease *In* International Congress Series No 193 Edited by CG Drake R Duvoisin Amsterdam Excerpta Medica Foundation Abstract #722 1969

3 Oehmichen M Cerebrospinal Fluid Cytology Philadelphia WB Saunders 1976 pp 60 141 142

4 Rautenbach M Der diagnostische wert liquorzytologischer Untersuchungen bei perinatalen Hirnblutungen Wiss Z Umm*boldt Univers Math Nat R 17 552 557 1968*

5 Rich JR *A survey of cerebrospinal fluid cytology Bull Los An*geles Neurol Soc 34 115-131 1969

6 Strassman G Formation of hemosiderin and hematoidin after traumatic and spontaneous cerebral hemorrhages Arch Pathol *47 205-210 1949*

Needle Aspiration of Metastatic Melanoma

Moshe Friedman, M.D.
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Vilasini Shanbhag, M.D.

Needle aspiration of nodal and extranodal metastases of malignant melanoma was evaluated in 74 patients. All patients had the initial diagnosis of malignant melanoma established on surgical biopsy and histologic sections. A 20 cc glass syringe and an 18 gauge needle were used, and the material obtained was stained by the Wright Giemsa technique. Adequate material was obtained and a cytologic diagnosis of malignant melanoma was made in 67 patients (90.5%). In seven patients (9.5%) the specimens contained nondiagnostic material. The diversity of cell types, cytoplasm staining affinity and pigmentation is described. Despite the high variability of the cytologic features encountered, malignant melanoma usually can be diagnosed on cytologic smears. Difficulties in recognizing the nonpigmented varieties are emphasized. The procedure was well tolerated and no complications were seen.

Regional or distant solid malignant melanoma metastases are frequently seen in an oncologic service. The diagnostic value of needle aspirates of such lesions has been reported only rarely. Zajicek[13], for instance, emphasized the high variability of cellular patterns in lymph node metastases. Effusion and exfoliative cytologic studies, on the other hand, are often reported in the literature. Hajdu and Savino[9] documented the high accuracy of the procedure on good smears obtained mainly from effusions. Malignant melanoma cells have also been reported in urine,[6,11] esophageal washings,[1,6] the oral cavity,[8] the vagina[2,7] and the endometrium.[10] In addition, Yamada et al[12] described the cytologic findings in 17 cases of malignant melanoma specimens which were obtained from oral cavity scrapings and autopsy imprints.

This paper reviews our experience with needle aspirates of extranodal and lymph node metastases in 74 patients seen at Roswell Park Memorial Institute from 1970 to 1978.

Materials and Methods

All patients included in this study had had a previous biopsy-proven malignant melanoma. The primary lesion was excised from the skin in 64 patients, from the anorectal area in one and from the vagina in one. In the remaining eight patients, the diagnosis was established on the basis of metastases originating from an unknown primary (Table I). The aspirated lesions included regional or distant enlarged and palpable lymph nodes in 48 patients, subcutaneous nodules located either around the previous surgical scar or at a distance from the original excision in 19 patients and deep-seated soft tissue masses or bony destructions in seven patients (Table II).

Needle aspiration was used to diagnose lesions detected during the follow-up. In patients with repeated relapses included in studies using various treatment modalities, measurable lesions were examined only by needle aspiration and preserved as parameters to evaluate the treatment response.

The technique described previously[3,4] was carefully applied so as to produce minimal trauma and obtain undamaged tumor cells undiluted with peripheral blood. A 20-ml glass syringe and an 18-gauge needle were used routinely. Accurate fixation of small mobile lesions, moderate vacuum applied to

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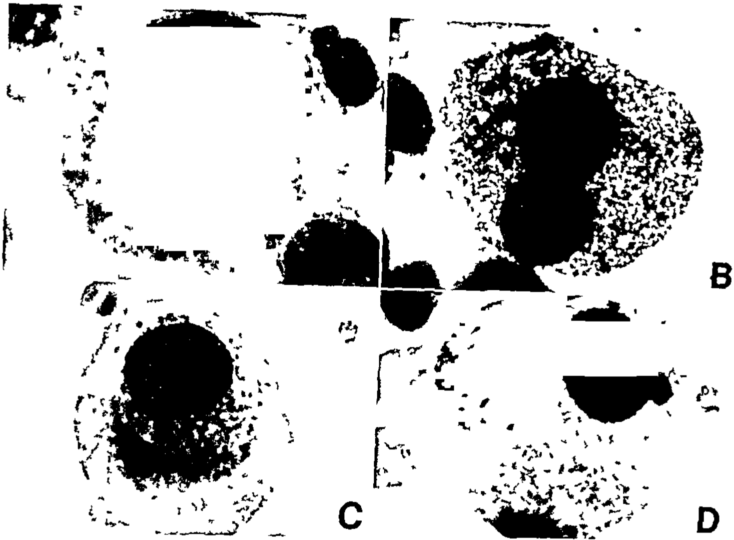


Figure 1
Round melanoma cells (× 1 400) Cells with well-defined borders abundant granulated cytoplasm eccentric nuclei and large blue nucleoli (A) Powder like melanin (B) Homogeneous-sized granules in a clear background (C) Bulky melanin granules (D) Powder like melanin

Table I *Primary Site of Melanoma Tumors*

Anatomic area	No of patients
Back	28
Extremities	20
Head neck	16
Vagina	1
Anorectal	1
Unknown	8
Total	74

Table II *Needle Aspiration of Metastatic Lesions*

Anatomic area	No of patients
Lymph nodes	48
Subcutaneous nodules	19
Soft tissue masses	5
Bone lytic lesions	2
Total	74

the syringe careful spreading of the material on glass slides and optimal staining time were the critical steps which required particular attention during the procedure Smears were air dried and stained according to the Wright-Giemsa technique Repeated aspirations during the same session or at 24 hour intervals were performed in cases which initially produced unsatisfactory material and in larger lesions to obtain material from different areas of the same tumor

Only lesions from which aspirates showed nondiagnostic material but whose clinical appraisals were in disagreement with the microscopic results were excised for histologic examinations

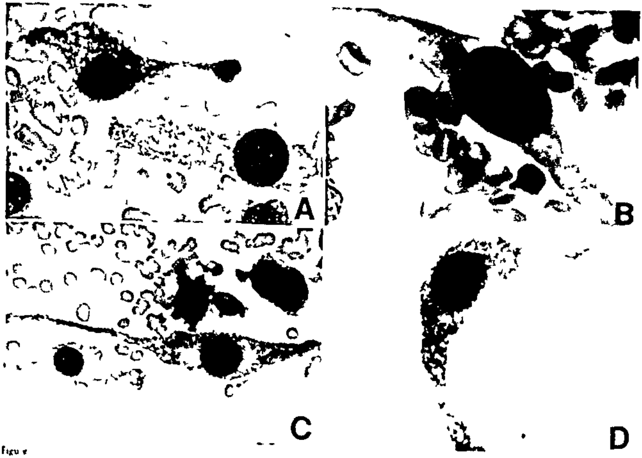
Results

The results of needle aspiration from solid metastases of malignant melanoma were analyzed in relation to (1) the rate of success in obtaining satisfactory material for diagnostic evaluation and (2) the micro scopic aspects seen on smears stained with the Wright-Giemsa technique. The sizes of the aspirated tumors are recorded in Table III. Most tumors were either less than 1 cm in diameter (27 cases) or between 1 and 2 cm (27 cases). Only five lesions were larger than 4 cm. Specimens containing diagnostic cellular material were obtained in 67 patients (90.5%). In seven patients (9.5%) nonneoplastic cells were obtained. The nondiagnostic specimens contained peripheral blood elements in three cases, cellular debris and degenerated cells from necrotic areas in two cases, fatty material in one case and reactive inflammatory cells in another case. Six of the lesions from which unsuccessful aspirations were performed measured less than 1 cm in diameter (two palpable lymph nodes and four skin or subcutaneous nodules). In two of these seven patients the lesions were excised, and the histologic sections showed malignant melanoma. In three patients follow-up of more than one year did not detect active disease at the site of the negative aspiration or elsewhere. Two patients were lost to follow up.

Table III *Size of Aspirated Lesions*

Size (cm)	No of patients
1	27
1 2	27
2-4	15
4	5
Total	74

In the 67 successful aspirations, the microscopic examination showed a high diversity of cytologic patterns with large variations of cell size, cell shape, nuclear changes, staining affinity and pigment distribution. The cell size varied from tumor to tumor and even within the same tumor. The median cell diameter was approximately 25μ to 30μ, with extremes from 10μ to 300μ. The *predominant cell type* found in most of the specimens was round or polyhedric (Figure 1). The typical melanoma tumor cell



Figure

Spindle-shaped melanoma cells (× 875). (A, C, D) The fusiform cell bodies are filled with black melanin granules. (B) The cell displays a dark blue cytoplasm, a granulated structure and a paranuclear micronucleus.

had sharply defined borders and an abundant cytoplasm often with a granulated texture which stained dark blue with Wright-Giemsa stain The round or oval nucleus was frequently eccentric and its chromatin displayed a clearly defined reticular network Other cells had a coarse appearing nucleus and the nuclei appeared hyperchromatic One or more blue nucleoli were frequently seen On specimens obtained from 11 cases spindle-shaped cells (Figure 2) were scattered among the round cells and in two of these cases fusiform cells were seen in larger numbers The spindle-shaped cells measured between 100μ and 400μ along their longitudinal axes In only five cases dendritic cells (Figure 3) were also found among other cell types Multiple arborizations with convoluted extremities arose from the main body of the cell and spread around it over a relatively large area

Mitotic figures were found in each case although they were few Cells in metaphase were most frequently encountered (Figure 4A) Among the atypical variants of mitotic figures the so-called C mitosis (Figures 4C and 4D) was often seen suggesting a disruption in the spindle fiber mechanism resulting in the random dispersion of the chromosomes in metaphase Hollow mitoses were also present occasionally (Figure 4B) Macrophages (melanophages) with ingested and degraded material mainly melanin pigment were seen in various numbers (Figures 5A and 5B) Melanophages with visible cell details showed degenerative changes with small pyknotic nuclei and a clear vacuolated cytoplasmic background in which the cellular and pigment debris was dispersed Nuclear vacuoles were seen rarely and only in three of our cases (Figure 5C) Giant mononucleated and multinucleated cells were present in almost every specimen either as scattered elements or as a dominant cell type (Figure 5D)

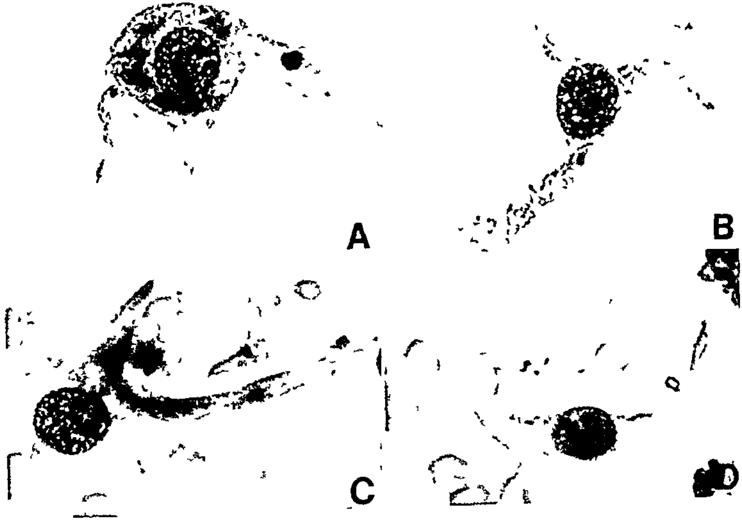


Figure 3
Dendritic melanoma cells (× 875) (A B) Multiple arborizations contain black melanin granules (C D) Arborizations contain deeply stained basophilic cytoplasm

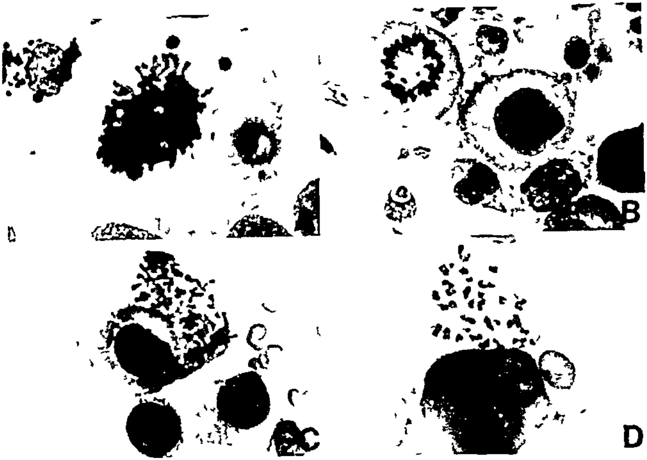


Figure 4
Melanoma cells in mitosis (× 875). (A) Metaphase. (B) Hollow metaphase. (C) C-mitosis with vacuolar area containing a giant melanin body. (D) C-mitosis.

The tumor cells were predominantly dispersed as single cells. Only on very rare occasions were cells found as clusters simulating orderly arranged and bounded cells (Figures 6A and 6C), rosettes (Figure 6B) or bundle-like, connected, spindle-shaped cells (Figure 6D).

Pigmentation, although a major diagnostic parameter, was not found as often as expected. Stratification of specimens according to the presence of cells containing melanin showed that between 50% and 90% of the cells were pigmented in 11 cases (Figure 7A); pigmented cells were mixed with more primitive pigment-free cells in 19 cases (Figure 7B); cells with deep blue homogeneous cytoplasm but no pigment were present in 26 cases (Figures 7C and 7D); and only tumor cells with pale whitish cytoplasm were found in 11 cases. The range of pigmentation in the cells varied from very heavy, completely obliterating the cell details (Figure 4D), to an empty cytoplasm with no structure and no staining affinity. Intermediate secretory stages containing various amounts of pigment were easily recognized. The black melanin pigment was seen either as a powder-like material (Figures 1A and 1D) accumulated in large amounts around the cell borders and spread out in the intercellular spaces or as bulky, round granules (Figure 1C) sometimes measuring 3μ to 5μ in diameter. On rare occasions cells were seen filled in with strikingly homogeneous-sized melanin granules (Figure 1B). The cytoplasmic processes emerging from the main body of the spindle or dendritic cells were often filled with melanin granules (Figures 2 and 3).

The background material consisted mainly of peripheral blood elements. In only five nodal aspirations was the tumor replacement of local tissue incomplete, resulting in scattered lymphoid cells among the tumor cells (Figures 7B, 7D and 8A).

The aspiration procedure was well tolerated, and

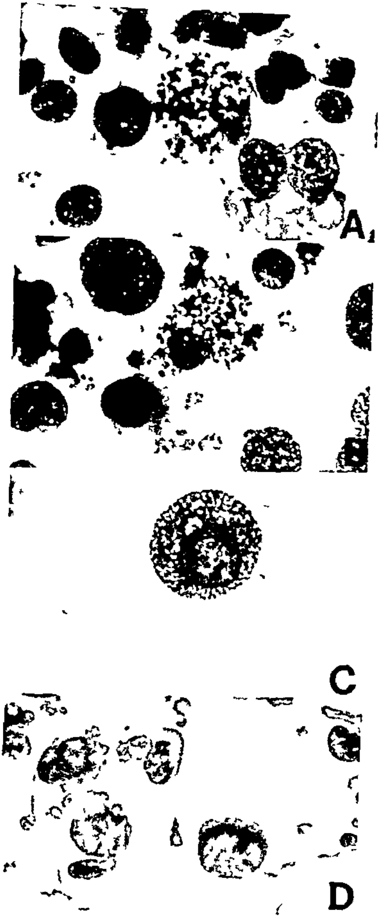


Figure 5
(A B) Melanophages with pyknotic eccentric nuclei and degraded material in the cytoplasm (× 875) (C) Melanoma cell with nuclear vacuole (× 875) (D) Giant mono and multinucleated bizarre melanoma cells (× 560)

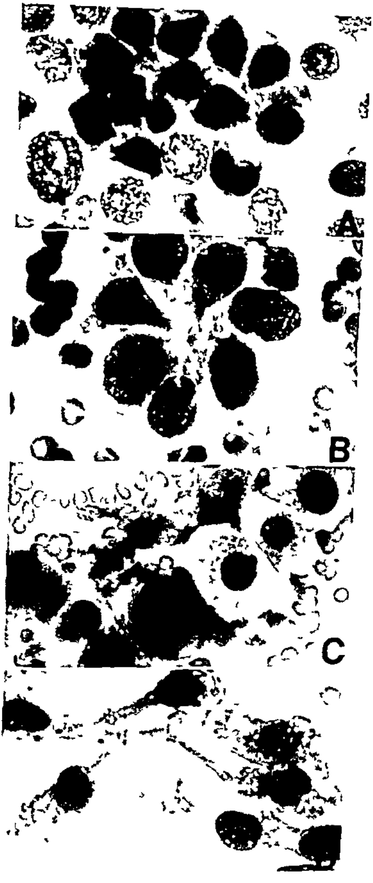


Figure 6
Uncommon distribution of melanoma cells (× 560) (A) Cluster of *cohesive cells with hyperchromatic nuclei and loose basophilic* cytoplasm (B) Rosette formation (C) Cord of tumor cells displaying various degrees of pigmentation
(D) Fusiform cells containing melanin

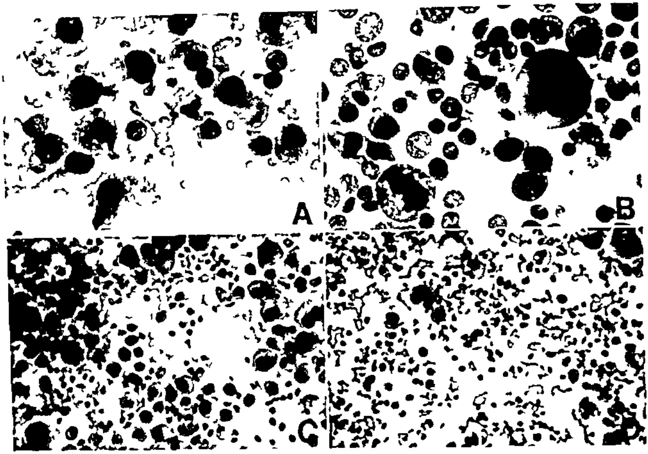


Figure 7

Pigmentation patterns in melanoma aspirates (A) Tumor cells display pigmentation in more than 90% of the cells (× 560) (B) Tumor consists of undifferentiated cells and scattered pigmented cells (× 560) (C) Tumor consists of cells with dark blue homogeneous cytoplasm with moderate pleomorphism and lymphoid cells in the background (× 350) (D) Tumor cells display a high degree of pleomorphism decreased staining quality and giant multinucleated bizarre cells (× 350)

the seeding of tumor cells along the needle tract was not documented

Discussion

In our experience needle aspiration of malignant melanoma lesions was often successful in procuring adequate material for cytologic investigations At least three factors contributed to the high rate of successful aspiration in malignant melanoma Since the local tissue is replaced almost entirely by the tumor rich specimens of tumor cells mixed with peripheral blood elements usually can be obtained Mild suction applied to the syringe during aspiration succeeds in dislocating the tumor cells from their environment due to their lack of cohesiveness Moderately large (1 cm to 3 cm) melanoma tumors were not associated in our experience with necrotic changes From most tumors well preserved tumor cells were obtained and the partial cell damage inherently related to the procedure was insignificant

In spite of the plurality of cytologic features of malignant melanoma several findings are common and may serve as guides in recognizing this tumor type on cytologic smears The presence of melanin pigment is the single most valuable morphologic marker of melanoma tumors Quite often the screening of the entire material to detect isolated pigmented cells is rewarding since it represents a valuable finding to justify the diagnosis of malignant melanoma in more difficult cases Singly dispersed cells were found in association with pleomorphic changes to various degrees Bi- and multinucleated cells and other bizarre elements were scattered among more uniform cells or dominated the entire picture

Nevertheless the difficulties in establishing a diagnosis on the basis of cytologic smears obtained from

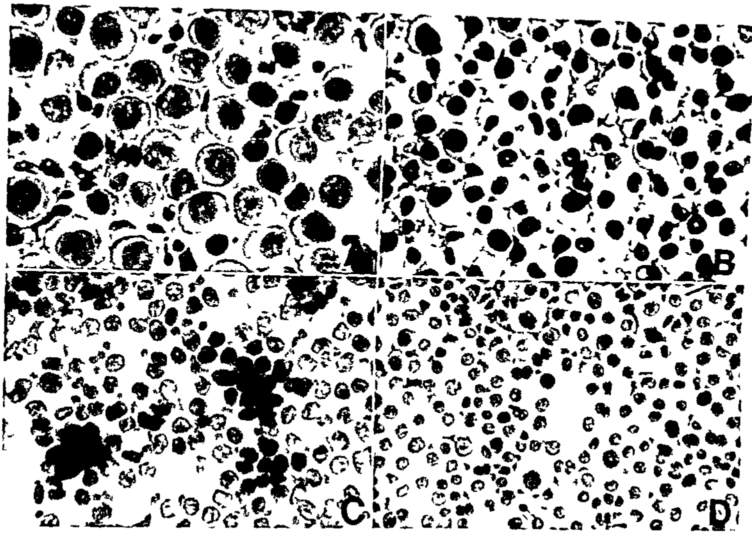


Figure 8
General view of melanoma aspirates (A) Single dispersed uniformly round melanoma cells with dark blue cytoplasm mimicking myeloma (× 560) (B) Pleomorphic cells with hyperchromatic nuclei and clear cytoplasm reminiscent of liposarcoma aspirates (× 560) (C) Cohesive tumor cells resembling undifferentiated epithelial tumors (× 350) (D) Homogeneously dispersed cells with pale to-clear cytoplasm resembling undifferentiated epithelial tumors (× 350)

nonpigmented melanoma lesions cannot be underestimated In our experience cases which displayed a close resemblance to multiple myeloma (Figure 8A) soft tissue sarcomas (Figure 8B) and undifferentiated epithelial tumors (Figures 8C and 8D) were encountered The close correlation with clinical and past histologic data the awareness of a previously excised and histologically diagnosed malignant melanoma and in particular cases a negative workup to rule out a second primary tumor justify the final diagnosis In more dubious cases excisional biopsy and electron microscopy are helpful in detecting cells with melanin pigment

Needle aspirations are justified by the need to have a tissue confirmation from each suspected lesion before treatment is applied Sometimes it is important to obtain such confirmation without excision since in repeatedly relapsed disease measurable lesions are true parameters which allow an objective evaluation of the response to the multiple combinations of drugs and radiation currently used in the management of melanoma

Acknowledgments

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References

1 Broderick PA Allegra SP Corvese N Primary malignant melanoma of the esophagus A case report Acta Cytol 16 159 164 1972

2 Ehrmann LR Younge PA Lerch VL The exfoliative cytology and histogenesis of any early primary malignant melanoma of the vagina Acta Cytol 6 245 254 196

3 *Friedman M Kim U Shimaoka K Panahon A Han T Stutz*man L Appraisal of aspiration cytology in management of Hodgkins disease Cancer (in press)

4 Friedman M Shimaoka K Getaz P Needle aspiration of 310 thyroid lesions Acta Cytol 23 194 03 1979

5 Hajdu SI Savino A Cytologic diagnosis of malignant melanoma Acta Cytol 4 3 0-327 1973

6 Johnson WD Koss LG Papanicolaou GN Seybolt JF Cytology of esophageal washings Cancer 8 951-957 1955

7 Linthicum CM Primary malignant melanoma of the vagina A case report Acta Cytol 15 159 164 1972

8 Medak H McGrew AE Burkalow P Jans RG Definitive cytopathologic characteristics of primary oral melanoma Oral *Surg* 27 237 246 1969

9 *Piva A Koss LG* Cytologic diagnosis of metastatic malignant melanoma in urinary sediment Acta Cytol 8 398-402 1964

10 Takeda M Diamond SM Demarco M Quinn DM Cytologic diagnosis of malignant melanoma metastatic to the endometrium Acta Cytol 22 503 506 1978

11 Woodard BH Ideker RE Johnston WW Cytologic detection of malignant melanoma in urine A case report Acta Cytol 22 350-351 1978

12 Yamada T Itou U Watanabe Y Ohashi S Cytologic diagnosis *of malignant melanoma Acta Cytol 16 70-76 1972*

13 Zajicek J Aspiration Biopsy Cytology Part I Supradiaphrag*matic Organs In Monographs in Clinical Cytology* Fourth volume Edited by GL Wied Basel S Karger 1974 p 119

XIIIth International Congress of the International Academy of Pathology and IVth World Congress of Academic and Environmental Pathology

The XIIIth International Congress of the International Academy of Pathology and the IVth World Congress of Academic and Environmental Pathology will be held in Paris France September 15-19 1980 English and French will be the official languages Direct inquiries to Christian Nezelof M D or Francis Jaubert M D Hôpital Necker Enfants Malades 149 rue de Sevres 75730 Paris Cedex 15 France

Differential Diagnosis of Malignant Lymphomas by Imprint Cytology

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The results of a study of 214 touch imprints of benign and malignant lymphoid disorders are presented The diagnostic criteria including the results of special stains are described for both benign proliferative disorders and malignant lymphomas Imprint cytology achieved an accuracy of 99% in diagnosing both benign and malignant conditions Specific sub [illegible] of malignant disorders was correctly made in 83% of non Hodgkin s lymphomas and in 66% of Hodgkin s lymphomas It is concluded that imprint cytology is a useful adjunct to the histopathologic diagnosis of lymphoid disease

The usefulness of imprint cytology and cellular smears as an adjunct to tissue diagnosis has been recognized for many years In conjunction with the frozen section the imprint or touch preparation is especially valuable in the preliminary diagnosis of lymphoproliferative disorders Sections of lymph nodes are often difficult to interpret whereas the cytologic method provides several advantages It (1) reduces artifactual distortion (2) enhances cellular detail (3) allows examination of a larger surface of the tissue and (4) provides a more rapid methodology with stained slides available in two minutes In our laboratory touch preparations are routinely made on fresh specimens submitted with possible neoplastic disease If the initial evaluation indicates a lymphoproliferative disorder a comprehensive workup of the tissue is undertaken Additional imprints are prepared for histochemical and immunologic evaluation and portions of the specimen are then processed for electron microscopy and histologic studies including enzymatic immunofluorescence and immunoperoxidase techniques

The classification of malignant lymphomas is undergoing continual revision Advances in immunologic techniques will undoubtedly produce additional criteria to subtype lymphomas on the basis of the cell of origin i e T or B cells Currently however with light microscopy different morphologic criteria are employed in the cytopathologic histopathologic diagnosis of malignant lymphomas

This study examined the cytologic characteristics of lymphoid diseases in various tissue specimens A one two minute quick-stain using a modified Papanicolaou technique is used on the alcohol-fixed imprints On selected tissue imprints special histochemical stains are employed Cytopathologic criteria for Hodgkin s and non Hodgkin s lymphomas are described below Benign lymphoproliferative disorders which may pose diagnostic difficulties are included

Materials and Methods

Imprints were made from lymph nodes and selected fresh tissue specimens requiring a rapid diagnosis which were sent to the Surgical Pathology Department of the Genesee Hospital During the period from July 1976 through December 1978 a total of 492 touch imprints were made Of them 235 were made from lymph nodes Metastatic disease was disclosed in 36 nodes and they were excluded from the study An additional 257 imprints were of organs representing the respiratory tract gastrointestinal tract geni

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tourinary tract endocrine system and central nervous system This study was limited to 214 imprints of benign and malignant lymphoid disease 199 were from lymph nodes and 15 from other tissue sites spleen bone marrow retroperitoneum lung skin skull and liver

The intact node was sectioned at the periphery and the cut surface used for imprints A clean microscopic slide was quickly and firmly touched to the cut surface of the tissue without smearing to better retain cell morphology Several imprints were made in this manner and placed immediately in a fixative For routine study the slides were fixed in 95% ethyl alcohol and stained with a modified Papanicolaou technique (Table I) If desired an imprint may be stained with the frozen section in a rapid hematoxylin and eosin stain (Table I) Following microscopic evaluation it was determined if additional stains would be helpful to verify a diagnosis In such cases imprints fixed in Carnoy s buffered methanol acetone or formalin vapors were utilized (Table II)

Results

Cytologic imprints were useful in differentiating between benign and malignant lymphoid disease and can be helpful in distinguishing among the various types of lymphomas Of the 214 imprints evaluated for this study only one specimen proved unsatisfactory in rendering a diagnosis The lymph node tissue in this case proved to be a lymphocyte-depleted Hodgkin s disease reticular type and the imprint was nearly acellular Two additional cases were misdiagnosed as malignant by imprint cytology One proved on permanent section to be a marked immune reaction with follicular lymphoid hyperplasia immunoblastic proliferation and plasma cell differentiation the cytologic diagnosis on the imprint was immunoblastic lymphoma Interestingly this patient developed an immunoblastic lymphoma four months later The second case early in our study was diagnosed by cytology as a diffuse poorly differentiated lymphocytic lymphoma but frozen and permanent sections revealed a lymphofollicular hyperplasia The numerous tingible body macrophages had been overlooked on the imprint

Table I *Stains for Routine Studies*

Rapid Papanicolaou stain			Rapid hematoxylin and eosin stain		
Immediate fixation in 95% ethanol for 30 seconds			Immediate fixation in 95% ethanol for 30 seconds		
1	95% ethanol	2 dips	1	95% ethanol	2 dips
2	H_2O	5 10 dips	2	H_2O	5 dips
3	Gill 3 hematoxylin	20 dips	3	Gill 3 hematoxylin	2 min
4	H_2O	10 dips	4	H_2O	5 dips
5	Saturated Li_2CO	5 dips	5	0 3% ammonia H_2O	until blue
6	H_2O	5 dips	6	H_2O	5 dips
7	95% ethanol	5 dips	7	Alcohol c eosin	5 dips
8	95% ethanol	5 dips	8	95% ethanol	4 dips
9	Orange G-6	5-10 dips	9	95% ethanol	4 dips
10	95% ethanol	5 dips	10	100% ethanol	4 dips
11	95% ethanol	5 dips	11	100% ethanol	4 dips
12	EA 50 or EA-65	5-10 dips	12	Xylene	5 dips
13	100% ethanol	5 dips	13	Xylene	until clear
14	100% ethanol	5 dips	14	Mount and coverslip	
15	100% ethanol	5 dips			
16	Xylene	5 dips			
17	Xylene	5 dips			
18	Mount and coverslip				

Table II *Special Stains for Diagnostic Confirmation*

Stain	Fixative	Diagnostic Importance	Slide Stability in Fixative	pH
Oil red O —	Cold concentrated formalin vapor	Neutral lipid (Burkitt s)	1 week	7.2
α naphthyl acetate esterase	Cold concentrated formalin vapor	Monocytes (histiocytic lymphoma)	3 days	7 4
Acid phosphatase	Cold buffered methanol acetone	"T"-cells (mycosis fungoides)	3 days	5.2 5 4
Tartrate resistant acid phosphatase	Cold buffered methanol acetone	Hairy cell leukemia	3 days	5.[illegible] 5 4
Methyl green pyronin	Cold Carnoy s	Immunoblasts and plasma cells (Burkitt s)	1 week	4 8

Modified from the original technique by the use of fast violet B as the coupler

A malignant cytologic diagnosis was accurately made in 75 of 76 cases (99%) and a benign cytologic diagnosis was correctly made in 136 of 138 cases (99%) Specific diagnoses as to the type of malignant disorder may be rendered in many instances as seen in Tables III and IV approximately 83% of non Hodgkin s lymphomas (including hairy cell leukemia and multiple myeloma) and 66% of Hodgkin s lymphomas were correctly subtyped Emphasis was not placed on being very specific as to the type of benign proliferative disorder once we were convinced of the nonmalignant nature of the lesion however in certain circumstances the correct interpretation was made as seen in Table V These lesions included reactive nodes hyperplasia granulomatous disease (necrotizing or nonnecrotizing) lymphadenitis immunoblastic reaction and sinus histiocytosis with an overall specific accuracy of 67 5%

The criteria for diagnosing certain lymphoproliferative disorders are outlined below Diagnostic accu

Table III *Accuracy of Specific Diagnoses in Malignant Non Hodgkin s Lymphomas*

	PDLLN	PDLLD	HL	WDLDD or CLL	BL	UNDIFF	MF	MM	HCL	*Total*
Cases correctly diagnosed	8	13	5	3	3	2	2	2	1	39
Total number of cases	9	15	8	4	3	3	2	2	1	47
Accuracy (%)	89	87	63	75	100	67	100	100	100	83 (weighted)

PDLLN = poorly differentiated lymphocytic lymphoma nodular type
PDLLD = poorly differentiated lymphocytic lymphoma diffuse type
HL = histiocytic lymphoma
WDLLD or CLL = well-differentiated lymphocytic lymphoma diffuse or chronic lymphocytic leukemia
BL = undifferentiated Burkitt s type lymphoma
UNDIFF = undifferentiated non Burkitt s type lymphoma
MF = mycosis fungoides
MM = multiple myeloma
HCL = hairy cell leukemia

Table IV *Accuracy of Specific Diagnoses in Malignant Hodgkin s Disease*

	HD NOS	HD MC	HD NS	*HD LP*	*HD LD*	Total
Cases correctly diagnosed	6	6	7	1	0	19
Total number of cases	6	9	11	1	2	29
Accuracy (%)	100	67	64	100	0	69 (weighted)

HD NOS = Hodgkin s disease not otherwise specified
HD MC = Hodgkin s disease mixed cellularity
HD NS = Hodgkin s disease nodular sclerosing
HD LP = Hodgkin s disease lymphocyte predominant
HD LD = Hodgkin s disease lymphocyte depleted
A correct interpretation of malignant was made in 75 of 76 cases including both Tables III and IV

Table V *Accuracy of Specific Diagnoses in Benign Proliferative Disorders*

	Reactive or hyperplasia	Lymphadenitis	Granulomas	Immunoblastic reaction	Sinus histiocytosis	Total
Cases correctly diagnosed	48	35	6	2	2	93
Total number of cases	54	43	14	3	14	128
Accuracy (%)	77	81	43	67	14	67 5 (weighted)

A correct interpretation of benign was made in 126 out of the 128 cases men may be enhanced by special stains and enzyme histochemical studies in selected cases

Benign Proliferative Disorders

Normal Germinal center cells include large and small cleaved cells zone of mature lymphocytes around the periphery of the germinal center few mitoses few tingible body macrophages follicular outline may be perceptible

Chronic Nonspecific Lymphadenitis Increased numbers of immature forms of lymphocytes mitoses common numerous macrophages

Lymphofollicular Hyperplasia Numerous tingible body macrophages mitotic figures may be prevalent a spectrum of immature and mature lymphoid cells from the germinal center many immunoblasts at different maturation levels (Figure 1)

Sinus Histiocytosis with Massive Lymphadenopathy[10] Emperipolesis[11] (lymphocytes engulfed within the cytoplasm of sinus histiocytes) (Figure 2)

Immunoblastic Reaction Large numbers of immunoblasts at different maturation levels plasmacytoid and plasma cells

Granulomatous Disease (Necrotizing or Nonnecrotizing) Epithelioid histiocytes multinucleated giant cells mature lymphocytes with or without necrotic debris

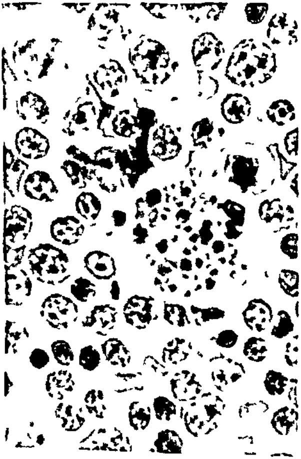


Figure 1
Lymphofollicular hyperplasia in lymph node Note tingible body macrophage and mature and immature lymphoid cells (Papanicolaou stain oil × 1 000)

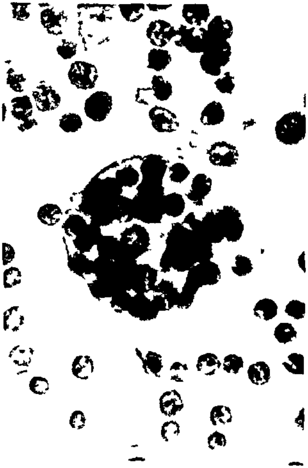


Figure 2
Sinus histiocytosis with massive lymphadenopathy emperipolesis from lymph node Observe mature lymphocytes within cytoplasm of sinus histiocyte (Papanicolaou stain oil × 1 000)

Malignant Proliferative Disorders

This classification is based on that of Berard and coworkers[2,3] (Table VI)

Nodular (Follicular) Lymphoma Poorly Differentiated Type Preponderance of the small cleaved lymphocyte with scanty cytoplasm cellular uniformity an occasional large mononuclear cell with vesicular nucleus and prominent nucleolus mitoses rare the small lymphocyte may have a single basophilic nucleolus located near the nuclear envelope the nodular formation may be evident on a low power scan of the imprint (Figure 3)

Diffuse Lymphoma Well Differentiated Lymphocytic Type Similar in appearance to chronic lymphocytic leukemia monotonous picture of small lymphocytes with round regular nuclei and scanty cytoplasm (Figure 4)

Diffuse Lymphoma Poorly Differentiated Lymphocytic Type Preponderance of larger lymphocytes round-oval nuclei with finely granular chromatin and some clearing one or more nucleoli nuclei may exhibit grooves cytoplasm is scanty (Figures 5 and 6)

Diffuse Lymphoma Mixed Type Cell population is approximately equally divided between the large and small lymphocyte types

Diffuse Lymphoma (True) Histiocytic Many cells with round-oval vesicular nuclei prominent nucleolus abundant cytoplasm characteristic cell markers for monocytes positive cytoplasm stains prominently and diffusely with α naphthyl acetate-esterase[14] *(Figure 7)*

Diffuse Lymphoma Undifferentiated Burkitt s Type[27] Large cells round-oval *nuclei chromatin* coarse and irregularly distributed in a clear parachromatin most nuclei contain multiple prominent *basophilic nucleoli cytoplasm is evident and con*tains vacuoles which stain positive for neutral lipid

Table VI *Classification of Lymphomas Adapted from Berard and Co workers*[1,2]

Nodular (follicular) lymphoma
- Poorly differentiated lymphocytic type
- "Histiocytic" type
- Mixed type

Diffuse lymphoma
- Well-differentiated lymphocytic type
- Poorly differentiated
  - Lymphocytic type
  - Immunoblastic type
  - Lymphoblastic type
- "Histiocytic" type

Undifferentiated Lymphoma
- Burkitt type
- Non Burkitt type

"T"-cell lymphoma
- *Mycosis fungoides*
- *Sézary syndrome*
- *Lymphoblastic lymphoma (some types)*

Hodgkin s lymphoma
- Lymphocyte predominant
- Nodular sclerosing
- Mixed cellularity
- Lymphocyte depleted

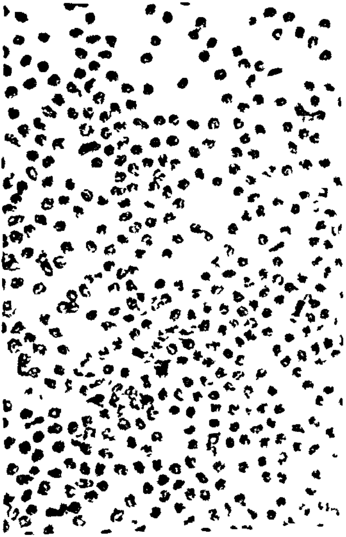


Figure 3
Nodular (follicular) lymphoma Poorly differentiated lymphocytic type in lymph node Note numerous small cleaved lymphocytes scanty cytoplasm and nucleolus (Papanicolaou stain × 40)

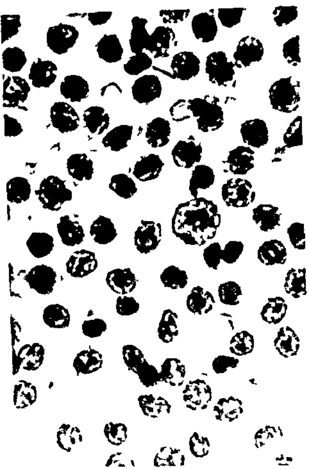


Figure 4
Diffuse lymphoma Well-differentiated lymphocytic type from lymph node Note preponderance of small round lymphocytes with scanty cytoplasm (hematoxylin and eosin oil × 1 000)

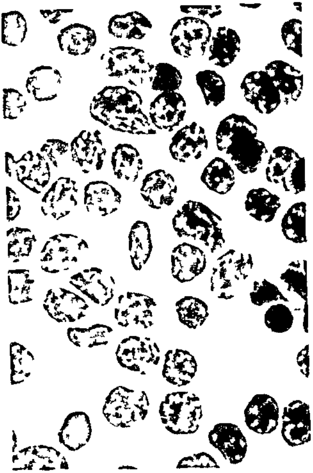


Figure 5
Diffuse lymphoma poorly differentiated lymphocytic type from skull Observe the larger lymphocytes with oval or round nuclei one or more nucleoli and scanty cytoplasm (hematoxylin and eosin oil × 1 000)

by oil red-O methyl-green-pyronin stain shows a uniform cytoplasmic pyroninophilia macrophages containing ingested debris distributed among the malignant cells giving the appearance of a "starry-sky" background (Figures 8 and 9)

Diffuse Lymphoma Mycosis Fungoides[1] Involves cutaneous tissue large cells with nuclei that are convoluted or cerebriform acid phosphatase stain reveals discrete granules within the cytoplasm of the neoplastic cells a finding helpful in identifying the "T-cell origin of this tumor (Figure 10)

Hairy Cell Leukemia (Leukemic Reticuloendotheliosis[11] Cells oval or indented nuclei may contain one or two nucleoli lacy chromatin and parachromatin well-defined cytoplasm may show irregular projections under oil immersion some cells may show positive staining for tartrate resistant acid phosphatase (TRAP) mitoses rare

Hodgkin s Disease Lymphocyte Predominant[11] Rare Reed Sternberg cells proliferation of lymphocytes and benign histiocytes occasional abnormal cells with large vesicular lobated nuclei and prominent nucleoli (Figure 11)

Hodgkin s Disease Nodular Sclerosing Reed Sternberg cells mononuclear and multilobulated variants of Reed Sternberg cells exhibiting lacunae and layering effect of cytoplasm

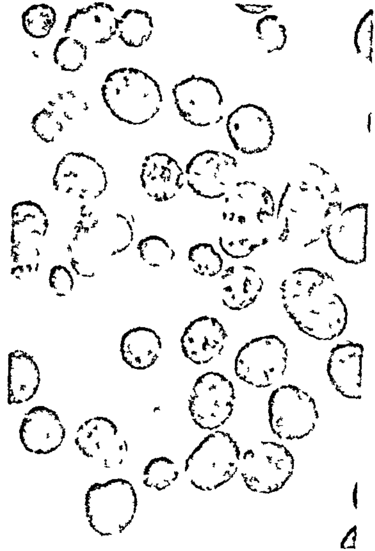


Figure 6
Diffuse lymphoma poorly differentiated lymphocytic type from colon similar to *Figure 5* *Note here the nucleoli and the finely* granular chromatin with clearing (Papanicolaou stain oil × 1 000)

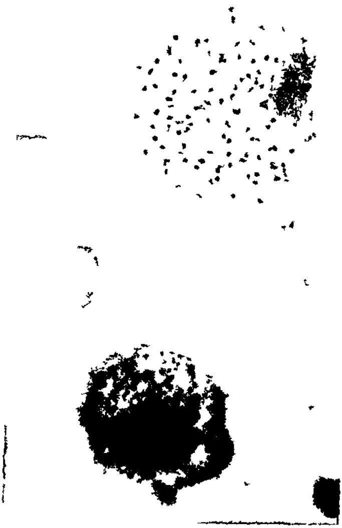


Figure 7
Histiocytic lymphoma Lymph node with cellular cytoplasm stained prominently and diffusely with α-naphthyl-acetate-esterase stain a monocytic cell marker (oil × 1 000)

Hodgkin s Disease Mixed Cellularity Numerous Reed-Sternberg cells eosinophils and plasma cells are usually seen

Hodgkin s Disease Lymphocyte Depleted Numerous Reed-Sternberg cells few lymphocytes plasma cells eosinophils and benign histiocytes

Discussion

Since 1927 when Dudgeon and Patrick[9] introduced a wet-film technique for the rapid diagnosis of new growths and inflammatory conditions the cytologic study of tissues has provided additional benefits in diagnostic pathology [2,8,10,11,18,22,24,26,27] The technique is particularly useful in the study of lymph nodes as was emphasized by Moore and Reagan in 1953[19] and again by Ultmann and co workers in 1958 [26]

Many fixatives including air drying and various staining techniques have been studied We found that immediate fixation in 95% ethyl alcohol followed by staining with a rapid modified *Papanicolaou* technique permits the enhanced microscopic examination of well preserved cells The excellence of the cellular morphology and the nuclear detail is crucial in making an accurate diagnosis

The classification of malignant lymphomas is continually being altered[1-3,5,7,13,24,25] as more is learned about the origin of the particular tumor i e *T* or *B* cells Various cell markers immunologic enzymatic or chemical [3,4,10,14,17,22,23] offer additional useful criteria as an aid in the complete workup for a diagno

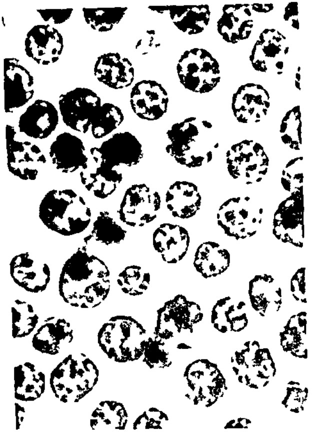


Figure 8
Burkitt s lymphoma Lymph node shows large round-oval cells with multiple prominent nucleoli and coarse irregularly distributed chromatin A small amount of cytoplasm is visible (Papanicolaou stain oil × 1 000)

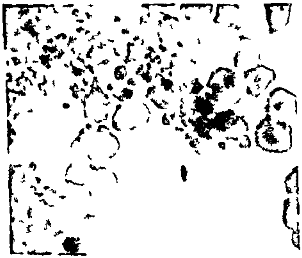


Figure 9
Burkitt s lymphoma Lymph node with cellular cytoplasm positive for neutral lipid (oil red O stain oil × 1 000)

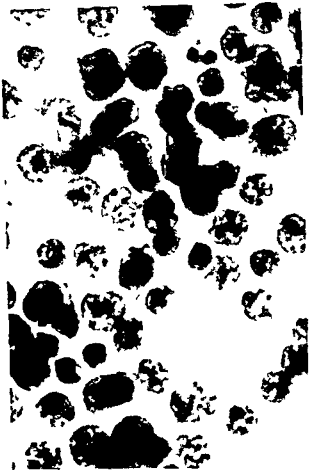
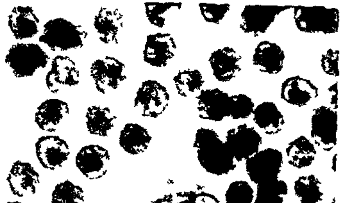


Figure 10
Mycosis fungoides from skin Observe the convoluted or cerebriform nuclei The cytoplasm is evident (toluidine blue stain oil × 1 000)

sis It is now believed that "true histiocytic" lymphomas are rare Most lymphomas originally categorized as histiocytic are actually lymphocytic in origin[4] True histiocytic lymphomas have cell markers similar to those of monocytes[22]

We utilized a modification of the classification of Berard and co workers[13] (Table VI) Several tumors may be studied further by histochemical methods While elaborate immunologic methods may not be available to every cytopathology or histopathology laboratory the above-described enzyme and chemical markers (Table II) may be utilized routinely The adaptability of imprints for these studies is described above

Imprint cytology is a valuable adjunct to the tissue diagnosis of lymphoid disease It is a quick easy re



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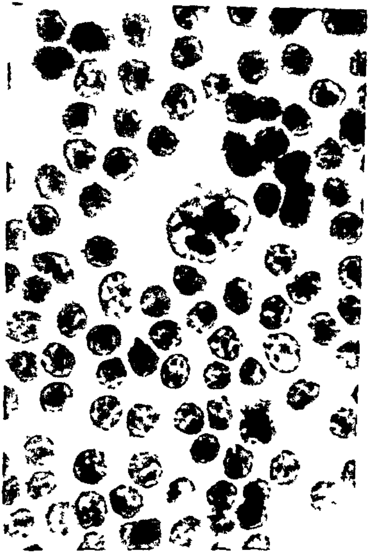

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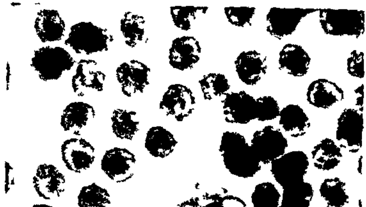

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Cytologic Monitoring of Cerebrospinal Fluid in the Treatment of Histiocytic Lymphoma Involving the Central Nervous System

Jill R Mayer, C T (ASCP), C M I A C
Carolyn W Watson, M D

This is a report of an unusual case in which cytologic monitoring played a key role in the six-and one half year survival of a 21-year old woman with disseminated histiocytic lymphoma involving the central nervous system At this writing the patient showed no further evidence of malignant disease After primary diagnosis remissions and exacerbations of the tumor were accurately detected by periodic cytologic examinations of 78 spinal fluid specimens Therapeutic measures included total brain irradiation implantation of an Ommaya reservoir and combination chemotherapy This case study demonstrates that cytologic examination of cerebrospinal fluid is an important method of monitoring the responses of tumors to therapeutic measures

Histiocytic lymphoma (HL) an aggressive tumor comprises about one third of all malignant lymphomas [3] It usually occurs between the ages of 15 and 45 with 5% to 6% under age 25 The overall survival rate 43% at one year drops to 20% at five years The five year survival rate for patients under 25 is only 12% [1 10] Central nervous system (CNS) involvement was demonstrated in 14% of 185 cases of HL seen at the New York Hospital in the past ten years [14] The natural development of this neoplasm has invariably led to death with only transient responses to chemotherapy Recently however new drugs and irradition used in combination are changing this gloomy prognosis [3]

In this report we present an unusual case of long survival of a young woman with HL with CNS involvement Periodic cytologic examination of the cerebrospinal fluid (CSF) accurately detected exacerbations and the effectiveness of therapeutic measures was *promptly evaluated*

Case History

A 21-year-old white woman was admitted to the New York Hospital in May 1972 with fever lymphadenopathy pruritis weight loss and low back pain radiating to both thighs Cervical lymph node biopsy revealed HL Other studies showed abnormal liver function skeletal lesions nodular defects of the ileum and massive retroperitoneal involvement She responded well to a combination of bleomycin vincristine streptonigrin and prednisolone and was discharged on maintenance cytoxan with periodic reinforcement by vincristine and prednisolone Six months later she developed headaches nausea and vomiting Even though an electroencephalogram and brain scan were negative a few tumor cells were found in her CSF

In March 1973 the patient was readmitted with persistent headache diminished hearing ataxia and papilledema Brain scan and electroencephalogram were still negative however multiple CSF specimens contained many pleomorphic lymphoma cells She

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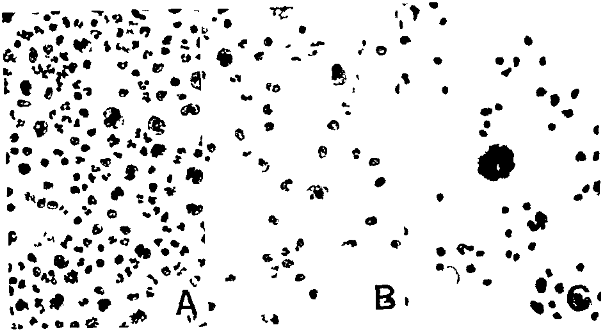

Figure 1
CSF These figures illustrate the patient s rapid response to intrathecal methotrexate and total brain irradiation (Papanicolaou stain × 400) (A) Day one pretherapy specimen Note markedly increased cellularity pleomorphism karyorrhexis pyknosis and polymorphonuclear leukocytes (B) Day three Note diminishing cellularity Nuclear lobulation multinucleation karyorrhexis and pyknosis are still prominent (C) Day six Note the multinucleated giant cell Cellularity karyorrhexis pyknosis and polymorphonuclear leukocytes are decreased

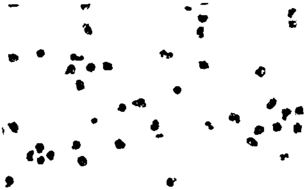

Figure 2
The patient s last abnormal CSF The malignant cells are more suggestive of lymphocytic lymphoma than histiocytic lymphoma (Papanicolaou stain × 400)

therefore received 1 800 rads of cobalt 60 to the whole brain and intrathecal methotrexate was initiated Her symptoms subsided and the number of malignant cells in the CSF dropped markedly (Figures 1A to 1C) By late May specimens were again negative Oral chemotherapy was discontinued because of decreased platelet and white blood cell counts The patient entered the hospital in June with a stiff neck vomiting and progressive dementia A few abnormal cells suggestive of lymphocytic lymphoma were found in her CSF Because long term intrathecal chemotherapy was anticipated an Ommaya reservoir was implanted[11,12] Several subsequent CSF specimens obtained via the Ommaya reservoir showed only minor reactive changes

Except for progressive bladder dysfunction the patient remained relatively well until February 1974 when malignant cells reappeared in her CSF Treatment with intrathecal Ara-C was initiated Soon thereafter only mild reactive changes were noted cytologically She was admitted several more times due to progressive cerebellar and cauda equina degeneration Additional CNS irradiation was given in March 1974 Tumor cells were last seen in her CSF in August 1975 They were more suggestive of lymphocytic lymphoma than of histiocytic lymphoma (Figure 2) In 1976 she had a colon conduit constructed for urinary diversion Her appendix removed at the same time contained a small asymptomatic carcinoid In February 1979 almost seven years after her initial hospitalization the patient was in complete remission and discontinuance of all chemotherapy was under consideration

iscussion

total of 78 CSF specimens was examined by the apanicolaou Cytology Laboratory All were prepared by the cytocentrifuge technique [15] stained by he Papanicolaou method and classified as follows 3 positive 10 suspicious 7 inconclusive 7 benign typia 34 normal and 7 virtually acellular An attempt was made to correlate therapeutic effect with mear pattern The degrees of cytologic abnormality nd cellularity of each specimen were compared The umber of cells per high-power field was estimated Ve found that the increase in cellularity was directly roportional to the degree of cytologic abnormality uspicious and positive specimens were further xamined to determine the size of malignant cells and he presence of prominent nucleoli All but one of the ositive specimens contained a variety of malignant ells ranging from small to large One positive and ive suspicious specimens contained small and nedium tumor cells Only small tumor cells were ound in the other five suspicious specimens Prominent nucleoli were noted in almost half of the specimens In the 17 instances in which CSF was obtained rom both the Ommaya reservoir and the lumbar rea the findings in each part of the specimen were ssentially the same

Figures 1A 1B and 1C illustrate the rapid response o combined treatment with intrathecal methotrexate and total brain irradiation The pretherapy specimen Figure 1A was obtained on the day methotrexate was initiated It contained many malignant cells polymorphonuclear leukocytes cellular debris and cells showing *karyorrhexis pyknosis* and occasional mitoses Cobalt 60 therapy was started the following day The next specimen Figure 1B taken two days after the first was somewhat less cellular Giant cells with prominent nucleoli multinucleation nuclear lobulation pyknosis and karyorrhexis were noted The specimen taken on the sixth day Figure 1C was similar but much less cellular

The phenomenon of karyorrhexis has been well documented [6, 9, 11] It may occur in patients with lymphomas and leukemias following irradiation and/or chemotherapy Similar changes have been found in pretreatment specimens from effusions and spinal fluids Spriggs[13] suggests that in HL some malignant cells outgrow their medium consequently dying cells with pyknotic or fragmented nuclei are interspersed among the viable tumor cells

Figure 3 summarizes on an annual basis the patient s therapeutic cytologic and clinical status The space allotted to each year is determined by the number of cytology specimens and the months in which they were obtained Clinical status and chemotherapy are similarly correlated Six drugs were used either alone or in combination systemically and two

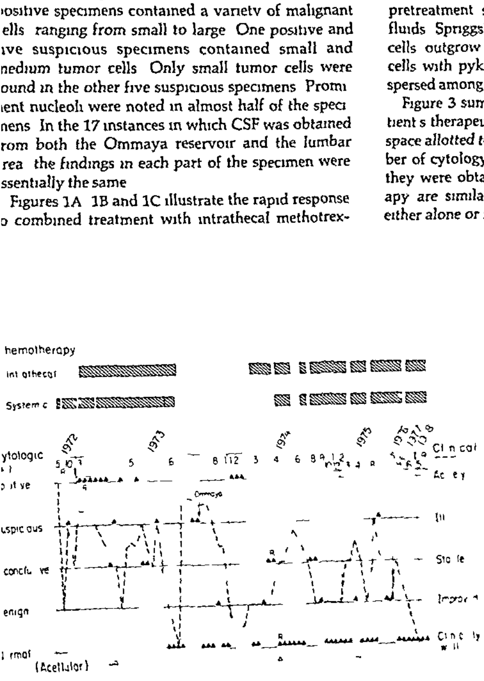

Figure 3
Cerebrospinal fluid cytology with therapeutic and clinical correlation Only the months in which specimens were obtained are indicated f each year (R = radiation — = clinical course ▲ = CSF cytology specimens △ = virtually acellular specimens)

were used singly intrathecally The clinical evaluations are subjective and represent the medical assessment of the patient s status at the time each specimen was obtained

A comparison of cytologic findings and clinical course shows interesting parallels and divergencies Although the patient was clinically well in May 1973 and January 1974 malignant cells were found in her CSF Conversely in the spring of 1974 in early 1975 and in May 1976 her cytology specimens were negative Though in clinical remission she was ill as a result of secondary complications including urinary tract infections dementia of uncertain etiology a neurogenic bladder due to tumor infiltration of the cauda equina and ataxia secondary to cerebellar degeneration

Both the disease process and therapeutic measures contributed to her morbidity D Angio[2] reported that posttreatment complications associated with irradiation and chemotherapy increased with combined therapy and were particularly prominent when irradiation and methotrexate were given together He concluded however There should be no shrinking from aggressive treatment when it is essential for cure Griffin[5] noted that when a lymphoma invaded the CNS corresponding neurologic abnormalities occurred The therapeutic results in his series suggested that an aggressive approach is worthwhile

This case substantiates the fact that cytologic examination of CSF is not only a reliable test for demonstrating meningeal involvement in patients with disseminated lymphomas but that it is also very useful for monitoring the course of the disease and therapeutic response[4,8,10] Despite the diverse forms of therapy used in treating this patient no specific cytologic reactions were detected

Acknowledgments

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References

1 Axtell LM Myers MH (editors) Recent Trends in Survival of Cancer Patients 1960 1971 Washington DC DHEW (NIH) 1974 p 28

2 D Angio GJ Complications of treatment encountered in lymphoma leukemia longterm survivors Cancer 42 1015 1022 1978

3 DeVita T Jr Canellos GP Chabner B Schein P Hubbard SP Young RC Advanced diffuse histiocytic lymphoma a potentially curable disease Lancet 1 248 250 1975

4 Drewinko B Sullivan MP Martin T Use of the cytocentrifuge in the diagnosis of meningeal leukemia Cancer 31 1331 1336 1973

5 Griffin JW Thompson RW Mitchinson MJ DeKiewiet JC Welland FH Lymphomatous leptomeningitis Am J Med 51 200-208 1971

6 Hajdu SI Hajdu EO Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors Philadelphia WB Saunders Co 1976 pp 75 85

7 Kolmel HW Atlas of Cerebrospinal Fluid Cells New York Springer Verlag 1976 pp 107 116

8 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Second volume Philadelphia JB Lippincott Co 1979 pp 974 979

9 Melamed MR The cytologic presentation of malignant lymphomas and related diseases in effusions Cancer 16 413-431 1963

10 Newell GR Measuring improved survival of cancer patients Ca 25 338 343 1975

11 Ommaya AK Cutaneous reservoir and pump for sterile access to ventricular cerebrospinal fluid Lancet 2 983 984 1963

12 Ratcheson RA Ommaya AK Experience with the subcutaneous cerebrospinal fluid reservoir N Engl J Med 279 1025 1031 1968

13 Spriggs AI Boddington MM The Cytology of Effusions Pleural Pericardial and Peritoneal and of Cerebrospinal Fluid Second edition New York Grune and Stratton Inc 1968 p 34

14 Tumor Registry The New York Hospital

15 Watson CW Bancroft J The use of the cytocentrifuge in a cytology laboratory (in preparation)

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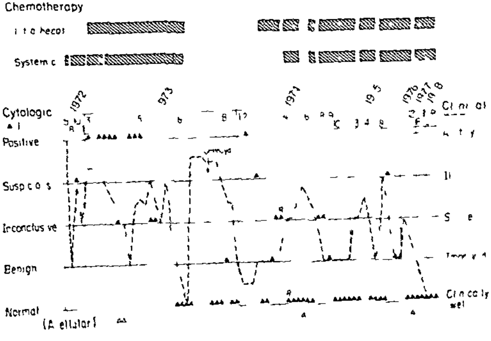

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A comparison of cytologic findings and clinical course shows interesting parallels and divergencies Although the patient was clinically well in May 1973 and January 1974 malignant cells were found in her CSF Conversely in the spring of 1974 in early 1975 and in May 1976 her cytology specimens were negative Though in clinical remission she was ill as a result of secondary complications including urinary tract infections dementia of uncertain etiology a neurogenic bladder due to tumor infiltration of the cauda equina and ataxia secondary to cerebellar degeneration

Both the disease process and therapeutic measures contributed to her morbidity D Angio[2] reported that posttreatment complications associated with irradiation and chemotherapy increased with combined therapy and were particularly prominent when irradiation and methotrexate were given together He concluded however There should be no shrinking from aggressive treatment when it is essential for cure Griffin[5] noted that when a lymphoma invaded the CNS corresponding neurologic abnormalities occurred The therapeutic results in his series suggested that an aggressive approach is worthwhile

This case substantiates the fact that cytologic examination of CSF is not only a reliable test for demonstrating meningeal involvement in patients with disseminated lymphomas but that it is also very useful for monitoring the course of the disease and therapeutic response [4 8 10] Despite the diverse forms of therapy used in treating this patient no specific cytologic reactions were detected

Acknowledgments

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References

1 Axtell LM Myers MH (editors) Recent Trends in Survival of Cancer Patients 1960 1971 Washington DC DHEW (NIH) 1974 p 28

2 D Angio GJ Complications of treatment encountered in lymphoma leukemia longterm survivors Cancer 42 1015 1022 1978

3 DeVita T Jr Canellos GP Chabner B Schein P Hubbard SP Young RC Advanced diffuse histiocytic lymphoma a potentially curable disease Lancet 1 248 250 1975

4 Drewinko B Sullivan MP Martin T Use of the cytocentrifuge in the diagnosis of meningeal leukemia Cancer 31 1331 1336 1973

5 Griffin JW Thompson RW Mitchinson MJ DeKiewiet JC Welland FH Lymphomatous leptomeningitis Am J Med 51 200-208 1971

6 Hajdu SI Hajdu EO Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors Philadelphia WB Saunders Co 1976 pp 75 85

7 Kolmel HW Atlas of Cerebrospinal Fluid Cells New York Springer Verlag 1976 pp 107 116

8 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Second volume Philadelphia JB Lippincott Co 1979 pp 974 979

9 Melamed MR The cytologic presentation of malignant lymphomas and related diseases in effusions Cancer 16 413-431 1963

10 Newell GR Measuring improved survival of cancer patients Ca 25 338 343 1975

11 Ommaya AK Cutaneous reservoir and pump for sterile access to ventricular cerebrospinal fluid Lancet 2 983 984 1963

12 Ratcheson RA Ommaya AK Experience with the subcutaneous cerebrospinal fluid reservoir N Engl J Med 279 1025 1031 1968

13 Spriggs AI Boddington MM The Cytology of Effusions Pleural Pericardial and Peritoneal and of Cerebrospinal Fluid Second edition New York Grune and Stratton Inc 1968 p 34

14 Tumor Registry The New York Hospital

15 Watson CW Bancroft J The use of the cytocentrifuge in a cytology laboratory (in preparation)

Urinary Cytology in Endometriosis of the Bladder

Volker Schneider, M D , M I A C
M J Vernon Smith, M D Ph D
William J Frable M D , F I A C

A case of endometriosis of the urinary bladder in a 27 year old black female is reported The cytologic histologic and clinical findings are presented and the differential diagnosis is discussed

Endometriosis is the aberrant growth of endometrial tissue *at ectopic sites* Permeation of endometrial glands into the myometrium (*adenomyosis interna*/endometriosis) is common and is found in 25% to 40% of all hysterectomy specimens Involvement of neighboring organs of the female genital tract (ovaries uterine ligaments rectovaginal and vesicovaginal septa cervix and vagina) is also frequently encountered

Endometriosis of organs not related to the female genital tract is seen rarely It has been reported to occur in the umbilicus laparotomy scars intestine appendix[15] lung[6] pleura[6,17] pelvic lymph nodes[11] kidney[5] ureter[13] and bladder[4,8,14,16] Recently we had the opportunity to observe a case of endometriosis involving the urinary bladder and were able to detect endometrial cells in a cytologic preparation of a urine sample

Case Report

This 27-year-old black female complained of voiding discomfort consisting of suprapubic pain bearing down on urination was particularly severe around menstruation There was no history of hematuria

The patient had a cesarean section in 1971 In 1975 endometriosis of the umbilicus was found and excised (Figure 1) At the time of the present admission she was under treatment for pelvic endometriosis Physical examination revealed a palpable mass in the posterior cervical area and cystoscopy showed a bulging mucosa of the bladder with a papillary lesion and several blue dome cysts of the posterior wall An intravenous pyelogram was unremarkable Laboratory values were within the normal range Voided urine and bladder washings were sent for cytology a biopsy was obtained during cystoscopy

Results

The cystoscopic biopsy demonstrated several intramural and submucosal foci of endometrial tissue in the wall of the urinary bladder (Figure 2) One of these foci extended to the surface of the bladder epithelium and caused ulceration (Figure 3) Focal secretory change of the endometrial glands was seen (Figure 4) Evidence of fresh and old hemorrhage with hemosiderin laden macrophages was present at several sites of the bladder wall

The voided urine specimen was fixed in 50% alcohol and two slides were prepared by the filter technique (Nuclepore) Two Nuclepore filter preparations were made from the fresh bladder washing specimen Microscopic examination revealed a cellu

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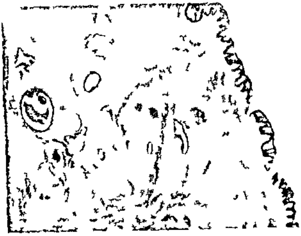

Figure 1
Endometriosis of the umbilicus (hematoxylin and eosin × 10)

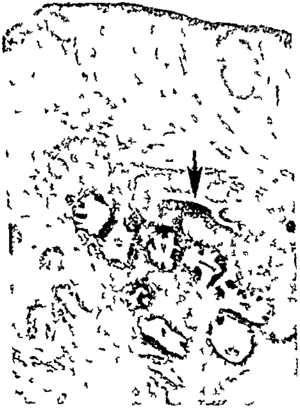

Figure 2
Endometriosis of the urinary bladder. Several endometrial glands are present, surrounded by dense stroma. A submucosal focus of urothelium (arrows) is intimately associated with the endometrial tissue (hematoxylin and eosin × 120).

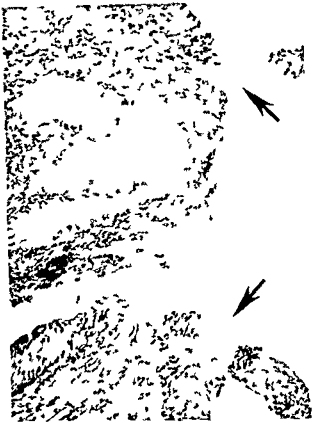

Figure 3
Endometriosis of the urinary bladder. The overlying urothelium (arrows) is disrupted; the endometrial tissue (center) exfoliates freely into the bladder lumen (right) (hematoxylin and eosin × 150).

lar specimen containing squamous cells, urothelial cells, and several groups of small dark cells which were considered to be endometrial cells. They occurred singly and in clusters, had a high nuclear-cytoplasmic ratio, and occasionally showed a cuboidal or cylindrical shape (Figures 5 and 6). Several clusters were present which resembled the double-contoured bodies occurring at the time of exodus in the postmenstrual vaginal smear. Hemosiderin-laden macrophages were seen in the bladder washing specimen.

Discussion

The first case of endometriosis of the urinary bladder was reported in 1921 by Judd.[8] A few reports of endometriosis of ureters[3] and of kidney[5] are on record. Up to 1966, 150 cases of bladder endometriosis were reported in the world literature,[4] establishing the bladder as the most common site of involvement

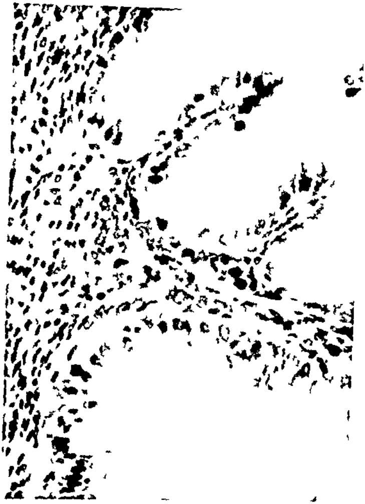

Figure 4
Some of the endometrial glands show papillary infoldings and secretory change (hematoxylin and eosin × 375)

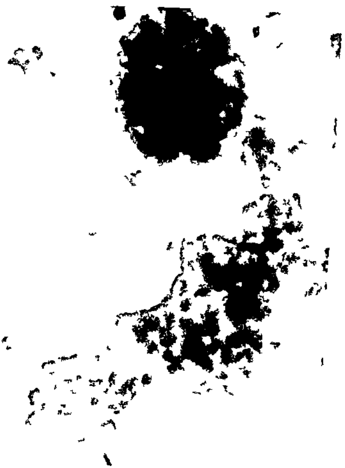

Figure 5
Clusters of endometrial cells are present in the voided urine (Papanicolaou stain × 600)

of the urinary tract by endometriosis A case of endometriosis of the bladder in a male patient treated by orchiectomy and estrogen for carcinoma of the prostate was reported by Oliker and Harris [12] It has been pointed out[1 18] that bladder biopsies in patients who developed cervico vesical fistulas after cesarean section mav erroneously be interpreted as endometriosis of the bladder

Classically the symptoms of endometriosis of the bladder are described to be hematuria which may be cyclic in character dysuria and suprapubic pain occurring in a patient who has a history of gynecologic surgery The disease is usually seen in women of childbearing age Cystoscopy reveals the presence of blue dome cysts most commonly involving the posterior wall

Since endometriosis rarely involves surfaces of mucosal membranes its presence is only occasionally detected by exfoliative cytology In the case presented here the endometrial tissue did reach the sur

Figure 6
Endometrial glandular structures are also seen in the bladder washing (Papanicolaou stain × 500)

face penetrated the urothelium and shed endometrial stromal and glandular cells into the urine (Figure 3)

Endometriosis has occasionally been detected by cytology at sites other than the urinary bladder Koss[10] reported the presence of endometrial cells in the spinal fluid of a 32 year-old woman with a history of debilitating headaches at the time of menstrual bleeding Granberg et al[6] diagnosed endometriosis of the lung and pleura by needle aspiration Wilhelm et al[17] found endometrial cells in a pleural fluid and Kanbour et al[9] presented the cytology of peritoneal washings with ovarian endometriosis

Cytologically the endometrial cells seen in the urine of this patient resembled for all intents and purposes those usually present in a vaginal smear Vaginal contamination in this case can be ruled out since endometrial cells were also present in the bladder washing specimen From a diagnostic point of view the unsuspected presence of small cells with a high nuclear-cytoplasmic ratio in a urinary specimen raises the possibility of malignancy e g poorly differentiated urothelial carcinoma lymphoma metastatic small cell carcinoma and sarcoma Follicular cystitis is a benign condition to be considered in the differential diagnosis However the marked uniformity of the cells and their occurrence in exodus type clusters should raise the suspicion of endometriosis of the bladder Of particular value for the differential diagnosis is the fact that endometriosis occurs almost exclusively in young women of childbearing age whereas most malignancies tend to occur in an older age group

Finally the possibility of malignancy arising in endometriosis has to be considered [2,7,11] Brooks and Wheeler[2] recently compiled 45 cases of adenocarcinoma arising in extragonadal endometriosis from the literature among which were four cases of carcinoma arising in endometrial foci of the urinary bladder It is of interest that among those 45 cases virtually all histologic types of carcinoma known to occur in the endometrium were seen including clear cell carcinoma squamous cell carcinoma endometrial stromal sarcoma and adenoacanthoma There was no evidence of malignancy whatsoever in our case

Prognosis of endometriosis of the urinary bladder depends upon the size of the lesion and occasionally requires partial cystectomy Treatment with antigonadotropin[3] or progesterone should be instituted initially

Acknowledgments

The authors thank Lois Paxson C T (ASCP) for her technical assistance

References

1 Borowski K Uterus Blasenfistel nach Sectio caesarea (Youssef Syndrom) unter dem Bild einer Harnblasen Endometriose Zentralbl Gynaekol 95 1684 1686 1973

2 Brooks JJ Wheeler JE Malignancy arising in extragonadal endometriosis A case report and summary of the world literature Cancer 40 3065 3073 1977

3 Dmowski WP Cohen MR Treatment of endometriosis with an antigonadotropin Danazol Obstet Gynecol 46 147 154 1975

4 Fein RL Horton BF Vesical endometriosis A case report and review of the literature J Urol 95 45 50 1966

5 Gauperaa T Stalsberg H Renal endometriosis Scand J Urol Nephrol 11 189 191 1977

6 Granberg I Willems JS Endometriosis of lung and pleura diagnosed by aspiration biopsy Acta Cytol 21 295 297 1977

7 Hyman MP Extraovarian endometrioid carcinoma Am J Clin Pathol 68 522 527 1977

8 Judd ES Adenomyoma presenting as a tumor of the bladder Surg Clin North Am 1 1271 1278 1921

9 Kanbour AI Klionsky B Romadella RM Cytology of peritoneal washings in ovarian endometriosis Acta Cytol 22 607 1978

10 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Philadelphia JB Lipp ncott 1979 pp 972 973

11 Koss LG Miniature adenoacanthoma arising in an endometriotic cyst in an obturator lymph node Cancer 16 1369 1372 1963

12 Ol ker AJ Harris AE Endometriosis of the bladder in a male patient J Urol 106 858-859 1971

13 Reddy AN Evans AT Endometriosis of the ureters J Urol 111 474-480 1974

14 Skor AB Warren MM Mueller EO Endometriosis of bladder Urology 9 689-692 1977

15 Uohara JK Kobara TY Endometriosis of the appendix Report of twelve cases and review of the literature Am J Obstet Gynecol 121 423-426 1975

16 Weinberg RW Vesical endometriosis Urology 11 72 73 1978

17 Wilhelm JL Scommegna A Catamenial pneumothorax Bilateral occurrence while on suppressive therapy Obstet Gynecol 50 227 231 1977

18 Youssef AF "Menouria following lower segment cesarean section A syndrome Am J Obstet Gynecol 73 759 767 1957

Vaginal Parasitosis

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Usha Saraiya, M D F I A C , F I C S
Madhura Paraskar, B Sc
Josephine Khokhawalla, B Sc

n two cases the ova of parasitic worms Ascaris lum ricoides *and* Enterobius vermicularis *were bserved in Papanicolaou-stained vaginal smears he characteristics of each type of ovum are de cribed*

From time to time reports have appeared in the literature of parasitic diseases evidenced by the presence of ova cysts or parts of parasites in cervicovaginal smears The incidence of worm infestations is very high in India particularly in the lower socioeconomic classes due to poor hygienic standards Careful screening of cytologic smears from patients in a general hospital is likely to reveal many interesting parasites This report deals with the finding of ova of parasites in Papanicolaou-stained smears from the vaginal pool

Case Reports

Case 1

A 45 year-old patient attended the hospital for copious foul-smelling discharge and backache Clinically she had a small friable lesion less than 1 cm on the posterior lip of the cervix The smears were taken from the posterior fornix Cytology revealed cells from an invasive carcinoma In one of the smears we found an unfertilized ovum of *Ascaris lumbricoides* or roundworm

Fertile eggs of *Ascaris* vary in size from 45μ to 75μ by 35μ to 50μ The typical egg is one celled and thick shelled The exterior is rough and mammillated in appearance although sometimes the rough exterior is missing Infertile eggs are considerably longer than fertile ones and lack interior cellular differentiation

The diagnosis of carcinoma was confirmed by biopsy The type of invasive carcinoma revealed by cytology and pathology was a large-cell nonkeratinizing epidermoid carcinoma grade II The diagnosis of roundworm infestation was confirmed by stool examination Treatment with Decaris (tetramisole) resulted in the expulsion of several worms Even at the time of Wertheim hysterectomy several dead roundworms were palpated in the intestine

To the best of our knowledge *Ascaris* eggs in the vaginal pool have not been reported so far This finding was almost certainly due to contamination with stools due to poor personal hygiene This patient also had a second-degree perineal tear so she had partial loss of sphincteric control

Case 2

D M was a *20-year-old patient* with threatened abortion Smears were requested for progesterone effect The smears were taken from the posterior fornix of the vagina and stained by the standard Papanicolaou method On one of the slides we found a typical ovum of the pinworm *Enterobius vermicularis*

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These eggs measure 55μ by 25μ i e the width is about half the length They stain eosinophilic with an orange red color The egg is flattened on one side Enclosed in the refractile shell are bright-orange staining larvae The shell is composed of an outer triple layer and inner monolayer The gravid female of *Enterobius* extrudes the eggs on the perineal skin and it is not unlikely that they can invade the vagina The intense pruritus associated with this disease can also lead to the deposition of eggs around the introitus These eggs can produce vaginitis and leucorrhea Cases have been reported of parasites migrating along the entire length of the genital tract and entering the peritoneal cavity through the fallopian tubes The background of the smear in this case did not show excess inflammatory response nor were many histiocytes present (Figure 1)

Discussion

Fentanes de Torres and Benitez Bribiesca of Mexico reported a series of 13 cases of pinworm ova in vaginal smears in 1973 [1] Langlinais in 1969 reported the finding of pinworm ova in smears [2] Among the parasites reportedly found in cervicovaginal smears are *Entamoeba histolytica Schistosoma hematobium Microfilaria* and pinworm To this list we now add our case of *Ascaris* ova

References

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2 Langlinais PC *Enterobius vermicularis* in a vaginal smear Acta Cytol 13 40-41 1969

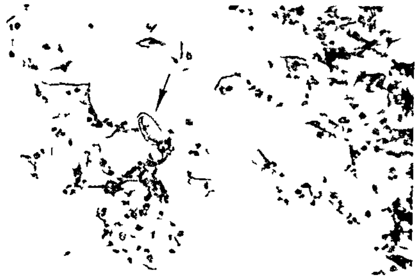

Figure 1
Ovum of pinworm *Enterobius vermicularis*

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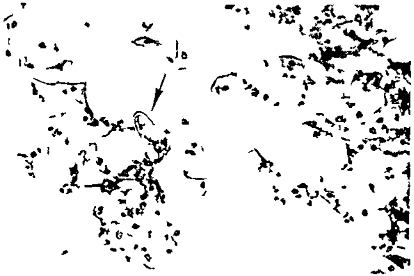

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Ovum of pinworm *Enterobius vermicularis*

Strongyloides stercoralis *in Papanicolaou-Stained Smears of Ascitic Fluid*

M Alejandra Avagnina M D
Boris Elsner, M D
Roberto M Iotti, M D
Ricardo Re, M D

Filariform larvae of Strongyloides stercoralis *were found by cytologic examination in Papanicolaou stained smears of ascitic fluid in a renal transplant recipient treated with immunosuppressive drugs The patient developed a fatal hyperinfection of* S stercoralis *produced by an autoinfective cycle of the parasite*

Infection with *Strongyloides stercoralis* is an endemic disease frequently found in tropical and subtropical areas up to 85% of the lower socioeconomic population can harbor the nematode in the small bowel Sporadic cases have been reported in countries with cooler climates [1,12]

S stercoralis can live for many years in the human body while producing either no symptoms or only vague gastrointestinal complaints diarrhea fever or transient pneumonitis Hyperinfection with widespread dissemination of the larvae may produce a severe and often fatal disease that is difficult to diagnose clinically This manifestation sometimes appears in a normal host Far more often it occurs in patients with other clinical conditions such as malnutrition or altered immunity[4] due to for example leukemia lymphoma leprosy systemic lupus erythematosus or treatment with high doses of corticosteroids or other immunosuppressive drugs [2,3] This paper describes a renal transplant patient treated with immunosuppressive drugs who was found to have extensive dissemination of the larvae Cytologic examination of the ascitic fluid showed the presence of the organisms

Case Report

The patient, a 33 year-old white man had advanced chronic renal insufficiency and was the recipient of a renal homograft He was put on immunosuppressive therapy with prednisone 40 mg per day and azathioprine 150 mg per day The patient had been born and lived in Formosa a province in the subtropical area of northern Argentina

Fifty days after the renal transplant the patient noticed the onset of epigastric pain nausea and vomiting with diarrhea and abdominal distension A maculopapular rash was observed on his head neck abdomen and lower limbs An upper gastrointestinal roentgenogram was interpreted as showing hemorrhagic duodenitis Routine laboratory data revealed a hematocrit of 37% and a white blood cell count of 9 000 per ml without eosinophilia Eight days after the onset of his symptoms the patient was operated on Surgical findings included a congested and dilated small bowel mesenteric lymphadenopathy and approximately 500 ml of bloody ascitic fluid that was submitted for cytologic analysis The smears stained with the Papanicolaou method showed red blood cells leukocytes lymphocytes some mesothelial cells and abundant filariform larvae of *S stercoralis* The larvae measured 400µm to 500µm in length and

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14μm to 16μm in diameter They had closed gullets (Figures 1 and 2) and curved blunt notched tails

In the immediate postoperative period the patient had several episodes of respiratory insufficiency with bronchial spasm and he died soon afterwards of cardiac arrest Just before death a chest X ray had shown diffuse infiltrates in both fields At autopsy ova of *S stercoralis* were found in the mucosa of the small bowel filariform larvae were found in the wall of the small (Figure 3) and large bowels as well as in the lungs associated with severe hemorrhage

Discussion

Strongyloides stercoralis is a nematode that dwells in warm and humid soils where the filariform (infecting) larvae can penetrate the bare skin and travel along the blood vessels towards the lungs Once there they penetrate the alveoli from where

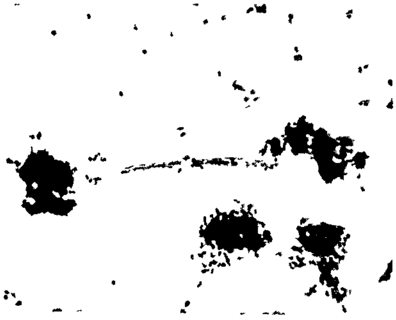

Figure 1
Smear of ascitic fluid showing filariform larvae of *Strongyloides stercoralis* (Papanicolaou stain × 100)

Figure 2
Higher magnification of the filariform larvae with details of its internal structure (Papanicolaou stain × 250)

Strongyloides stercoralis *in* *Papanicolaou-Stained Smears of Ascitic Fluid*

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14μm to 16μm in diameter They had closed gullets (Figures 1 and 2) and curved blunt notched tails

In the immediate postoperative period the patient had several episodes of respiratory insufficiency with bronchial spasm and he died soon afterwards of cardiac arrest Just before death a chest X ray had shown diffuse infiltrates in both fields At autopsy ova of *S stercoralis* were found in the mucosa of the small bowel filariform larvae were found in the wall of the small (Figure 3) and large bowels as well as in the lungs associated with severe hemorrhage

Discussion

Strongyloides stercoralis is a nematode that dwells in warm and humid soils where the filariform (infecting) larvae can penetrate the bare skin and travel along the blood vessels towards the lungs Once there they penetrate the alveoli from where

Figure 1
Smear of ascitic fluid showing filariform larvae of *Strongyloides stercoralis* (Papanicolaou stain × 100)

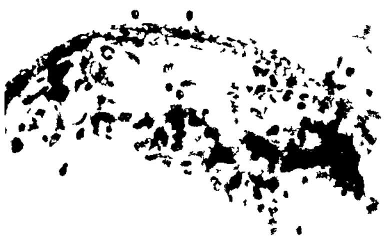

Figure 2
Higher magnification of the filariform larvae with details of its internal structure (Papanicolaou stain, × 250)

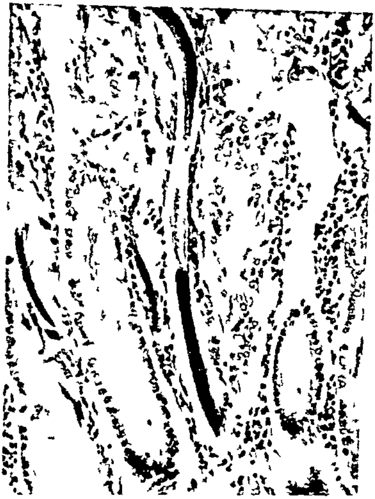

Figure 3
Section of the small bowel mucosa taken at autopsy in which larvae of *Strongyloides stercoralis* are seen (hematoxylin and eosin × 100)

through the trachea and larynx they find their way towards the gastrointestinal tract The adult worms become localized in the small bowel and they lay eggs that mature into the rhabditiform larvae that are found in feces In the soil the rhabditiform larvae may mature into free living adults that continue to reproduce or may metamorphose into filariform larvae that cause infection Rhabditiform larvae may also metamorphose into filariform larvae in the host s small bowel and penetrate the wall of the bowel or the perianal skin producing an autoinfective cycle that perpetuates the infection [1] Under several conditions as mentioned above in the introduction the autoinfective cycle can assume massive proportions The presence of filariform larvae in the ascitic fluid and lungs in our patient demonstrated the existence of a hyperinfective manifestation [11] produced by a massive autoinfective cycle Such systemic strongyloidiasis has rarely been described in renal transplant recipients treated with immunosuppressive drugs having been reported only by Fagundes et al in Brazil[4] and by Scoggin and Call[10] and Scowden et al[11] in the United States

Although larvae have been found and described in sputum[5,10] and their existence in ascitic fluid mentioned briefly [6,7] no description of the morphologic features of *S stercoralis* in the ascitic fluid is available in the cytologic literature From a purely morphologic standpoint *S stercoralis* must be differentiated from *Ancylostoma duodenale* which is not indigenous to the United States and from *Necator americanus* and *Ascaris lumbricoides* These other nematodes do not have the blunt and notched tail of *S stercoralis* the tails of their filariform larvae are sharply pointed Neither *Ascaris* nor the hookworms produce autoinfective cycles and massive infection as does *S stercoralis* [1]

Cytology can contribute to the initial diagnosis of infection due to *S stercoralis* during the life of the patient either by sputum examination[5,10] or as in this case by finding the larvae in the ascitic fluid If a duodenal aspirate is studied larvae can be observed in 90% of the cases [8] In approximately 80% of the cases larvae of *S stercoralis* can also be found in fresh stool although often requiring elaborate concentration procedures This diagnosis and confirmation is very important because life-saving therapy with thiabendazole can be instituted in some cases it can result in a cure [11]

Immunosuppressed patients from areas where *S stercoralis* is endemic should undergo stool examination to rule out infection with this parasite and the possibility of a massive autoinfection such as the one described in this report [1]

References

1 Blumenthal DS Intestinal nematodes in the United States N Engl J Med 297 1437 1439 1977

2 Civantos F Robinson MI Fatal strongyloidiasis following corticosteroid therapy Am J Dig Dis 14 643-648 1969

3 Cruz T Reboucas G Rocha H Fatal strongyloidiasis in patients receiving corticosteroids N Engl J Med 275 1093 1096 1966

4 Fagundes LA Busato O Brentano J Strongyloidiasis Fatal complication of renal transplantation Lancet 2 439-440 1971

5 Kenney M Webber CA Diagnosis of strongyloidiasis in Papanicolaou-stained sputum smears Acta Cytol 18 270 273 1974

6 Liepman M Disseminated *Strongyloides stercoralis* A complication of immunosuppression JAMA 231 387 389 1975

7 Lintermans JI Fatal peritonitis An unusual complication of *Strongyloides stercoralis* infestation Clin Pediatr 14 974-978 1975

8 I ralta NR Rodriguez MA *Strongyloides stercoralis* larvae in gastric and duodenal aspirates Acta Cytol 22 61-62 1978

9 Purtilo DT Meyers WN Connor DH Fatal strongyloidiasis in immunosuppressed patients Am J Med 56 488-493 1974

0 Scoggin CH Call NB Acute respiratory failure due to disseminated strongyloidiasis in a renal transplant recipient Ann Intern Med 87 456 458 1977

11 Scowden EB Schaffner W Stone WJ Overwhelming strongyloidiasis An unappreciated opportunistic infection Medicine 57 527 544 1978

12 Smith SB Schwartzmann M Mencia F Blum EB Krogsted D Nitzkin J Healy GR Fatal disseminated strongyloidiasis presenting as acute abdominal distress in an urban child J Pediatr 91 607 609 1977

Diagnosis of Strongyloides stercoralis *in Sputum Cytology*

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Cheryl Strinden C T (ASCP)

A unique case of hyperinfection with Strongyloides stercoralis *in an apparently immunosuppressed host is reported The organism in various stages of maturation was initially detected by routine sputum cytology Papanicolaou stained smears from scrapings of jejunal and colonic mucosa provided specific confirmation of the parasite Postmortem examination also showed massive tissue and vascular invasion in the intestine with cerebral and pulmonary dissemination*

Sputum cytology is a widely used noninvasive and inexpensive screening procedure[13] It may provide the earliest diagnostic evidence of premalignant and malignant lesions[7,11,15] With experience benign tumors as well as infectious conditions due to viruses fungi and parasites may be identified[7,11] Among the parasitic infections amebiasis and strongyloidiasis have been previously diagnosed by a routine Papanicolaou-stained sputum examination[9,11,15]

This paper describes a case of hyperinfection with *Strongyloides stercoralis* in which the initial detection of the worm was made in a routine sputum cytologic examination

Case Report

A 60-year-old Caucasian male was admitted to Hines VA Medical Center with an abrupt onset of focal seizures and right hemiparesis The patient was a known alcoholic and had never been out of the United States Computer tomographic scan showed multiple brain lesions consistent with abscesses Abnormal laboratory results included anemia mild leukocytosis and eosinophilia and guaiac positive stools The causative agent thought to be *Staphylococcus* was never isolated Because of the nonspecific investigative data he was placed empirically on broad-spectrum antibiotics and an antituberculous regimen Steroid treatment was also instituted for the management of the increased intracranial pressure No improvement was recorded The patient later developed a diffuse bronchopneumonia which similarly was unresolved despite prompt therapy His deteriorating physical condition precluded neurosurgical intervention

The patient lapsed into a terminal coma A routine sputum cytology revealed acute and chronic inflammatory cells and some parasites The latter predominantly larvae were elongated spindle-shaped slender and orangeophilic and measured approximately 250μ to 280μ in length (Figure 1) In a clearly visualized larva a stout small buccal cavity and a long esophagus were seen An occasional fully distended egg with a young larva about to emerge from the membranous confines was noted (Figure 2) Adult female worms about five times longer than the free larvae were occasionally noted (Figure 3)

No specific treatment was initiated because the patient died a day after the cytologic recognition of the parasite Pertinent postmortem findings included *Strongyloides stercoralis* parasitemia and widespread candidiasis The entire small and large intestine displayed acute linear and serpiginous ulcerations with

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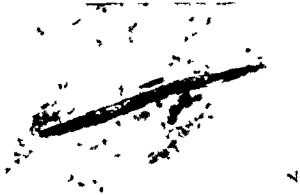

Figure 1
A sputum smear showing a long slender and orangeophilic larva (Papanicolaou stain × 200)

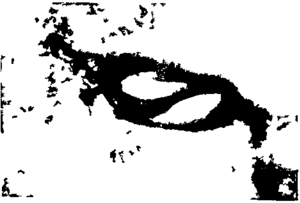

Figure 2
Sputum with a fully developed young larva within an egg (Papanicolaou stain × 3 0)

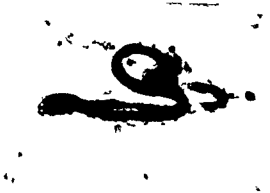

Figure 3
Parasitic adult female worm in the sputum (Papanicolaou stain × 00)

tissue and vascular invasion from numerous worms in various stages of maturation Examination of the Papanicolaou-stained smears from scrapings of the jejunal and colonic mucosa demonstrated the characteristic rhabditiform and filariform larvae uniformly embryonated eggs and parasitic female and male adult worms of *S stercoralis* (Figures 4 and 5) The organism together with *Candida albicans* complicated the abscessed pneumonic and cerebral lesions In addition septic myocardial and splenic infarcts were caused by *Candida*

Discussion

A middle aged man who was apparently immunosuppressed [13,14] presented with brain abscesses In

Figure 4
A Papanicolaou-stained scraping smear of the jejunal mucosa demonstrating two filariform larvae of *Strongyloides stercoralis* (× 800)

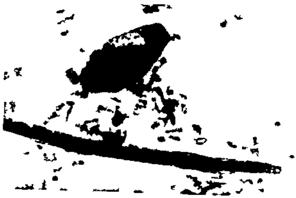

Figure 5
An embryonated egg and a larva of *Strongyloides* from the colonic mucosal scraping (Papanicolaou stain × 200)

ıddıtion to the antibiotics and antituberculous regi nens steroid therapy was instituted for more than a nonth in an attempt to control the increased intra- ranial pressure Eventually two infectious compli ations were diagnosed *Strongyloides* hyperinfec ion and candidemia Both opportunistic organ sms[13,16] were microscopically documented in the erebral and pulmonary lesions The *Strongyloides* nassively reproduced in the small and large intestine vith tissue and vascular invasion Septic myocardial nd splenic infarcts were attributed to the *Candida*

This case is interesting from several aspects First he parasite was originally discovered by a routine putum cytologic examination Second, various tages of *Strongyloides* life cycle were recognized in single sputum specimen They included young habditiform larvae about to break free of the mem- ranous confines of the egg many filariform larvae nd rare adult female worms This observation might e explained by contamination of the sputum with astric and duodenal contents however there was o notation of terminal vomiting with the request for ytologic screening The other possible explanation ould be an extraintestinal maturation and comple on of the life cycle of *S stercoralis* possibly in the ing in this overwhelmingly infected and immuno ippressed patient Third the absence of a history of avel outside the United States makes this patient a are autochthonous instance of strongyloidiasis oc irring in a temperate climate [3,5,9,10] Finally no stool amination for parasites was ever done despite the osinophilia guaiac-positive feces, negative workup ata and puzzling clinical syndrome

Although *S stercoralis* is basically a tropical and ibtropical parasite it does occur in about 1% to 4% the population in the southern United States [3,9] ie infection is more commonly found in adults an children [3,10] Its spread in mental and penal institutions with warm moist and congested environ- ents is notorious [3,5,9,10] While poor hygiene defi tely increases parasitism, prevention of defecation to the soil and adequate treatment of cases are the ly effective means of eradicating the disease [3,10]

The infection with *Strongyloides* commences with rval penetration of the skin The larva then invades e cutaneous vascular channels migrates through e right side of the heart and lung capillaries capes into the alveoli where it matures into an dolescent worm [3,6] and finally ascends the respirato- tract to the glottis From the glottis the larva is vallowed thus reaching its ultimate residence in the testine The entire length of small and large intestine may be involved but the duodenum and jeju num are the favored sites [1,6,8,10,12,14,16]

The predominant symptom usually is diarrhea which may be refractory to treatment [3,10] Mild case of strongyloidiasis are frequently asymptomatic [3,5,1 The parasite can survive in the intestine for many years [1,8,9,13] and in a host with poor hygienic habit the so-called autoinfection may be observed [1,3,6,9,10,16] In autoinfection the larvae mature to the infective filariforms in the perianal region and initiate a new skin penetration either therein or in the buccal mucosa following ano-oral contamination [2,3,5,10] This is followed by the usual migration via the vascular heart lung pathways and the establishment of another generation of organisms A further internal infection termed *hyperinfection* occasionally occurs with impaired shedding of the rhabditiform larvae in the feces or with immunosuppression [3,5,8-13,15] The infective filariform larvae are produced in the intestine and start a mucosal penetration into the mesenteric veins The host develops a severely exaggerated form of parasitism with dissemination of the organism to almost every organ [1,3,5,8,16]

The diagnosis of strongyloidiasis is based on the findings of the rhabditiform larvae in the feces duodenal aspirates and rarely sputa [2,3,5,6,9,10,12,14,16] The embryonated eggs filariform larvae and adult worms are also distinctive when found [2,3,5,9,10,16,17] Of utmost value is a high index of suspicion for the presence of this helminthic infection[3] in patients

Acknowledgments

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References

1 Brown HW Perna VP An overwhelming *Strongyloides* infection JAMA 168 1648 1651 1958

2 Eveland LK Kenney M Yermakov V Laboratory diagnosis of autoinfection in strongyloidiasis Am J Clin Pathol 63 421-425 1975

3 Faust EC Russell PF Jung RC Craig and Faust s Clinical Parasitology Eighth edition Philadelphia Lea & Febiger 1970 pp 284 294

4 Gage JC Case of *Strongyloid s intestinalis* with larvae in human strongyloidiasis Am J Trop Med 20 259-375 19 0

5 Grove DI Warren KS Mahmonud AAF Algorithms in the diagnosis and managemen of exotic diseases III Strongyloidiasis J Infect Dis 131 755-759 1975

6 Kenney M Webber CA Diagnosis of strongyloidiasis in Papanicolaou-stained smears Acta Cytol 18 270-273 1974

7 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Second volume Philadelphia JB Lippincott 1979 pp 586 587

8 Kyle LH McKay DG Sparling HJ Jr Strongyloidiasis Ann Intern Med 29 1014 1042 1948

9 Marcial Rojas RA Pathology of Protozoal and Helminthic Diseases New York Robert E Krieger Publishing Co 1975 pp 711 733

10 Markell EK Voge M Diagnostic Medical Parasitology Fourth edition Philadelphia WB Saunders 1976 pp 255 260

11 Naib ZM Exfoliative Cytopathology Second edition Boston Little Brown and Co 1976 pp 231 294

12 Peralta NR Rodriguez MA *Strongyloides stercoralis* in gastric and duodenal aspirates Acta Cytol 22 61-63 1978

13 Purtilo DT Meyer WM Conner DH Fatal strongyloidiasis in immunosuppressed patients Am J Med 56 488-493 1974

14 Rivera E Maldonado H Velez-Garcia E Grillo AJ Malaret G Hyperinfection syndrome with *Strongyloides stercoralis* Ann Intern Med 72 199 204 1970

15 Schumann GB Colon VF Sputum cytology Am Fam Physician 19 81-84 1979

16 Scowden EB Schaffner W Stone WJ Overwhelming strongyloidiasis Medicine 57 527 544 1978

17 Smith JW Gutierrez Y Medical parasitology *In* Todd Sanford Davidson Clinical Diagnoses and Management by Laboratory Methods Sixteenth edition Second volume Edited by JB Henry Philadelphia WB Saunders 1979 pp 1773 1783

The Morphometry of the Sudan-III-Positive Granules in the Cytoplasm of the Human Amniotic Epithelium

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Seventy nine human amnions from different gestational ages were studied by new morphometric methods The Sudan III positive granules in the cytoplasm of the amniotic epithelial cells were first observed in the reflected amnion from the second trimester of normal gestation During the third trimester of normal gestation the number of cytoplasmic granules increased remarkably with gestational age not only in the reflected amnion but also in the placental amnion The number of granules was generally related to the increasing rates of anucleated cells and binucleated cells in the amniotic epithelium during the third trimester of normal gestation During the third trimester of gestation the number of granules was greater in the distant reflected amnion with its less adequate blood supply than in the placental amnion A vast accumulation of the granules in the entire amnion was observed in cases of fetal death in which blood supply had ceased

It is suggested therefore that the progressive accumulation of Sudan III positive cytoplasmic granules in the human amniotic epithelium during the third trimester of normal gestation is the result of cell degeneration

The increasing accumulation of Sudan III *positive* granules in the cytoplasm of the human amniotic epithelial cells with gestational age has been recorded[2] [7] [10] *but the biologic meaning of this change has not* been elucidated thoroughly The quantitative analysis of this change is the purpose of this paper

Materials and Methods

Seventy nine human amnions from different gestational ages were used in this study The 69 normal cases included 14 in the first trimester 8 in the second trimester and 47 in the third trimester of gestation There were ten cases with intrauterine fetal death composed of five cases in the first trimester three in the second trimester and two in the third trimester of gestation The first trimester was defined as less than 20 completed weeks of gestation the second trimester as over 20 completed weeks but less than 28 and the third trimester as 28 completed weeks and *over according to WHO standards*[11]

The normal cases in the first trimester and the early half of the second trimester were selected from legal *abortions induced by curettages and Porro s operations* Some cases in the latter half of the second trimester and in the early third trimester were selected from premature vaginal deliveries in which no neonatal complications arose for at least 24 hours after

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delivery The other normal cases in the third trimester were selected from term and postterm vaginal deliveries in which no neonatal complications arose for at least seven days after delivery

The cases of fetal death in the first trimester were selected from both complete abortions and incomplete abortions with the amniotic sac including the dead fetus and those in the second and third trimesters were selected from cases of artificial delivery within several days after cessation of fetal vital signs

Discoid portions of fresh membrane about 2 cm in diameter were excised from both the placental amnion and the reflected amnion The fresh membranes were stretched in new devices made of plastic double rings The stretched standardized specimens were fixed with 10% formalin and stained with Sudan III and with hematoxylin The connective tissue in the outer layer was removed and the specimens were mounted in glycerin The histologic structures of the epithelial layer of the amnion were clearly observed under the microscope and could be distinguished from other stromal layers of the amnion

In order to measure the nuclear and cytoplasmic features of the epithelial cells framed squares measuring 10 000μm² were projected on the TV monitor of an automatic microscopic particle measurement computer apparatus composed of the πMC System (Millipore Ltd Bedford Massachusetts) and a Vanox microscope (Olympus Optical Co Ltd Tokyo Japan) The total number of nuclei in the unit area was counted automatically and the numbers of anucleated cells binucleated cells and Sudan III positive granules were determined for the unit area The total number of cells in the unit area was obtained by subtracting the number of binucleated cells from the total number of nuclei and adding the number of anucleated cells The number of Sudan III positive granules per cell was obtained by dividing the number of granules by the total number of cells in the unit area The rate of anucleated cells or binucleated cells was represented as the percentage ratio of such cells to the total number of cells in the unit area

To show clearly the morphometric results the averages of measured values of each specimen were plotted on the dispersion diagrams at intervals of four or eight gestational weeks The values of the placental amnion and the reflected amnion were calculated separately

Results

Sudan III positive granules were observed in the cytoplasm of the amniotic epithelial cells The cytoplasmic granules were first observed in the reflected amnion in all cases of second trimester normal gestation Their number in the reflected amnion and in the placental amnion increased remarkably with gestational age During the third trimester of normal gestation up to 20 fat granules per cell could be observed (Figures 1 to 3) In most normal cases the number of granules in the reflected amnion was greater than in the placental amnion In most cases of fetal death especially in the cases in the second and third trimesters of gestation the number of granules in both amnions was almost identical to the number in normal cases at the end of gestation and was greater than in normal cases of the same gestational age (Figure 1)

The anucleated cells in the amniotic epithelium

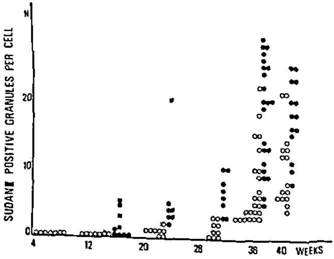

Figure 1
The accumulation of Sudan III positive granules in the cytoplasm of the normal amniotic epithelium in relation to gestational age Note the vast accumulation of granules in the cases with fetal death (○ = placental amnion normal case □ = placental amnion fetal death case ● = reflected amnion normal case ■ = reflected amnion fetal death case)

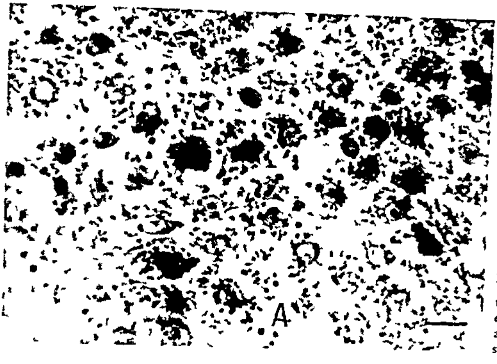

Figure 2
The epithelial nuclei and sudanophilic cytogranules of the normal placental amnion from delivery at the 40th week of gestation "A" indicates an anucleated cell (Sudan III and hematoxylin stain × 450 scale = 20μ)

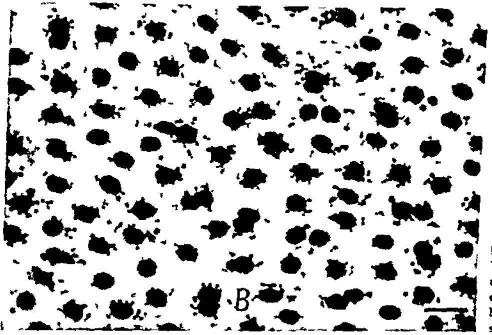

Figure 3
The epithelial nuclei and sudanophilic cytogranules of the normal placental amnion from delivery at the 36th week of gestation "B" indicates a binucleated cell (Sudan III and hematoxylin stain × 450 scale = 20μ)

were first observed in the second trimester of normal gestation The rate of anucleated cells in both amnions increased remarkably with gestational age during the third trimester of normal gestation In most normal cases this rate was higher in the reflected amnion than in the placental amnion In most cases of fetal death the rate of such cells in both amnions was similar to that in normal cases at the end of gestation and was higher than in the normal cases of the same gestational age (Figure 4)

During the third trimester of normal gestation there was a significant correlation between the number of Sudan III positive granules per cell and the number of anucleated cells (Figure 5)

The binucleated cells in the amniotic epithelium were first observed in some cases in the second trimester of normal gestation The rate of binucleated cells of both amnions increased during the third trimester of normal gestation In most normal cases however the difference in the number of such cells between the reflected amnion and the placental amnion was not very significant In most cases of fetal death the number of such cells in both amnions was not higher than in normal cases (Figure 6)

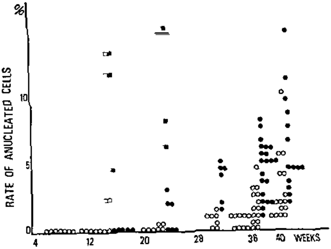

Figure 4
The increasing rate of anucleated cells of the normal amniotic epi thelium in relation to gestation l age Note the high frequency of anucleation of cell in the cases with fetal death (O = placental amnion normal case ☐ = placental amnion fetal death case ● = reflected amnion normal case ■ = reflected amnion fetal death case)

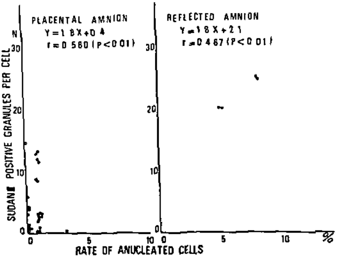

Figure 5
Significant correlation between the number of Sudan III positiv cytogranules per cell and the frequency of anucleation in the normal amniotic epithelium from the third trimester of gestation

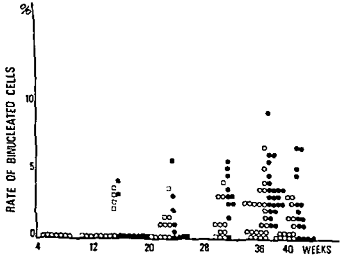

Figure 6
The increasing rate of binucleated cells of the normal amniotic epithelium in relation to gestational age Note the high frequency of binucleation of cells in some cases with fetal death (O = placental amnion normal case ☐ = placental amnion fetal death case ● = reflected amnion normal case ■ = reflected amnion fetal death case)

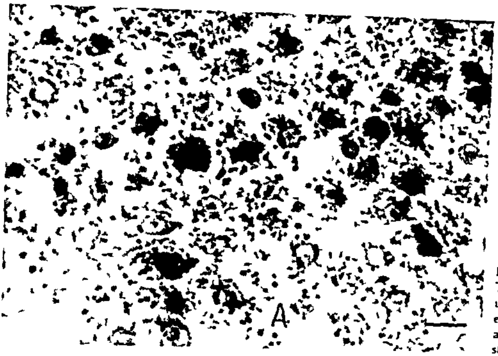

Figure 2
The epithelial nuclei and sudanophilic cytogranules of the normal placental amnion from delivery at the 40th week of gestation A indicates an anucleated cell (Sudan III and hematoxylin stain × 450 scale = 20µ)

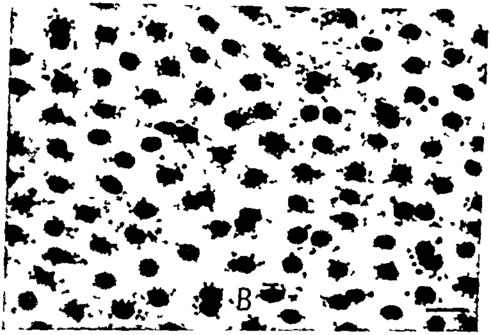

Figure 3
The epithelial nuclei and sudanophilic cytogranules of the normal placental amnion from delivery at the 30th week of gestation B indicates a binucleated cell (Sudan III and hematoxylin stain × 450 scale = 20µ)

were first observed in the second trimester of normal gestation The rate of anucleated cells in both amnions increased remarkably with gestational age during the third trimester of normal gestation In most normal cases this rate was higher in the reflected amnion than in the placental amnion In most cases of fetal death the rate of such cells in both amnions was similar to that in normal cases at the end of gestation and was higher than in the normal cases of the same gestational age (Figure 4)

During the third trimester of normal gestation there was a significant correlation between the number of Sudan III positive granules per cell and the number of anucleated cells (Figure 5)

The binucleated cells in the amniotic epithelium were first observed in some cases in the second trimester of normal gestation The rate of binucleated cells of both amnions increased during the third trimester of normal gestation In most normal cases however the difference in the number of such cells between the reflected amnion and the placental amnion was not very significant In most cases of fetal death the number of such cells in both amnions was not higher than in normal cases (Figure 6)

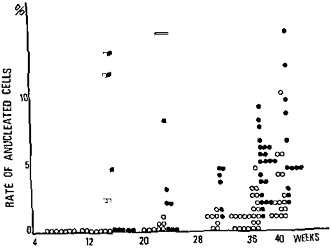

Figure 4

The increasing rate of anucleated cells of the normal amniotic epithelium in relation to gestational age Note the high frequency of anucleation of cells in the cases with fetal death (○ = placental amnion normal case □ = placental amnion fetal death case ● = reflected amnion normal case ■ = reflected amnion fetal death case)

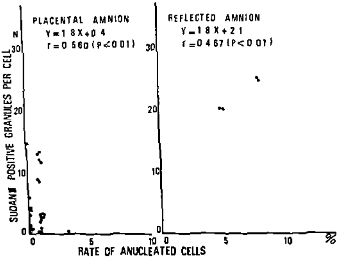

Figure 5

Significant correlation between the number of Sudan III positive cytogranules per cell and the frequency of anucleation in the normal amniotic epithelium from the third trimester of gestation

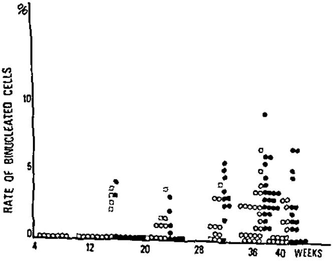

Figure 6

The increasing rate of binucleated cells of the normal amniotic epithelium in relation to gestational age Note the high frequency of binucleation of cells in some cases with fetal death (○ = placental amnion normal case □ = placental amnion fetal death case ● = reflected amnion normal case ■ = reflected amnion fetal death case)

Discussion

The hypotheses concerning the accumulation of Sudan III positive granules in the cytoplasm of the human amniotic epithelial cells can be generally divided into two groups One group of observers has suggested that the Sudan III positive granules reflected either active fat secretion into the amniotic fluid[2,3,10] or other cell activity[4,6] Armstrong et al[2] suggested on the basis of ultrastructural and biochemical studies that the granules were a secretory product and the source of vernix caseosa Based on time-lapse studies of amnion cells *in vitro* the same authors suggested that the sudanophilic vacuoles were not the result of cell degeneration Other observers have suggested that the granules were indeed the result of cell degeneration[5,7] Harima[5] indicated on the basis of histochemical studies that Sudan III positive granules in the amniotic epithelial cells represented cell degeneration rather than an active metabolic process His conclusion was based on the rapid increase in the number of granules toward the end of gestation when the chorionic and decidual tissue degenerated the greater accumulation of granules in the reflected amnion than in the placental amnion possibly corresponds to the relative insufficiencies of blood supply

Most of the past hypotheses concerning the accumulation of the Sudan III positive granules are fundamentally dependent upon the qualitative studies of the granules and disregarded other morphologic cellular changes The morphologic findings concerning not only the cytoplasmic granules but also other cellular changes may shed some light on this problem In this study the accumulation of the cytoplasmic sudanophilic granules was evaluated by newly designed morphometric methods[13] and was correlated with nuclear changes which were simultaneously counted

During the second trimester of normal gestation the fat granules in the amniotic epithelial cells were first observed to be accompanied by anucleated and binucleated cells After the third trimester of normal gestation there was a good correlation between cells with fat granules and the anucleated and binucleated cells Binucleated cells which are found frequently in aged humans and animals may represent cell degeneration[1,11] Anucleated cells are dead cells[8,9] The Sudan III positive granules were observed more in the reflected amnion than in the placental amnion toward the end of the third trimester of gestation the blood supply to the distant reflected amnion at that time may become less sufficient than to the placental amnion[5] Moreover the vast accumulation of fat granules in the whole amnion observed in the fetal death cases may be considered a result of cell degeneration caused by an absence of blood supply

Therefore we suggest that the progressive accumulation of Sudan III positive cytoplasmic granules in the human amniotic epithelium toward the end of the third trimester of normal gestation is the result of cell degeneration

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References

1 Andrew W Changes in the nucleus with advancing age of the organism Adv Gerontol Res 1 87 107 1964

2 Armstrong WD Wilt JC Pritchard ET Vacuolation in the human amnion cell studied by time lapse photography and electron microscopy Am J Obstet Gynecol 102 932 9 8 1968

3 Bondi J Zur Histologie des Amnionepithels Zentralbl Gynaekol 35 1073 1076 1905

4 Bourne GL The Human Amnion and Chorion London Lloyd Luke Ltd 1962 p 29

5 Harima N The clinical and histopathological study of inflammation in the fetal membrane placenta and umbilical cord Acta Obstet Gynaecol Jpn 16 127 136 1964 (in Japanese)

6 Hata R The histopathological study of the human amnion J Hokkaido Gynaecol Soc 7 16 28 1956 (in Japanese)

7 Maruyama J The electron microscopic study of the human amnion epithelium Acta Obstet Gynaecol Jpn 24 187 197 1972 (in Japanese)

8 Ogawa K Differentiation and growth of cells *In* New Cytology Edited by S Senoo et al Tokyo Asakura Shoten 1965 p 592 (in Japanese)

9 Pollicard A Bessis M Elements de Pathologie Cellulaire In Japanese translation by E Yamada M Imai Tokyo Asakura Shoten 1972 p 157

10 Pritchard ET Armstrong WD Wilt JC Examination of lipids from amnion chorion and vernix Am J Obstet Gynecol 100 289 298 1968

11 Tauchi H Sato T Some micromeasuring studies of hepatic cells in senility J Gerontol 17 254 259 1962

12 World Health Organization Expert Group on Prematurity Final Report Geneva WHO Techn Rep Ser 27 1950 (quoted from World Health Organization ICD RE 74 4 1974)

13 Yoshimura S Yoshida Y Nishimura T The morphometry of aging of the human amniotic epithelium *In* Proceedings of the Sixth International Congress of Cytology Japan 1977 p 342

Studies of the Distribution of Abnormal Cells in Cytologic Preparations

I Making the Smear with a Wooden Spatula

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A model system of 85% exfoliated normal human cervicovaginal squamous cells 15% exfoliated rodent tumor cells and acellular viscous mucus like material was used to investigate the distribution of cells on smear preparations Smears were made by the same individual with wooden spatulas using traditional smearing motions The presence or absence of normal squamous cercivovaginal cells and of tumor cells in ten randomly distributed areas was recorded Smears made with counterclockwise circular motions contained the largest number of tumor cells and those with zigzag strokes contained the least Independent of the motion employed the largest number of tumor cells was found in ring 9 and the lowest in rings 4 and 10 Some of the factors influencing the accumulation of tumor cells in certain areas of the slide may be unequal pressure of the spatula during smearing variations in the angle between the instrument and the surface of the slide and the displacement of tumor cells to certain areas of the preparation by overlapping smearing

The usual purpose of cervicovaginal cytologic examinations is to detect abnormal cells These examinations are time consuming and require the concentrated attention of highly skilled technicians or cytopathologists Despite an enormous amount of time and capital in attempts to mechanize this process there have been no studies regarding the possible factors influencing the distribution of atypical cells in smears

A well known characteristic of blood smears is that larger cells are found at the periphery of the smear while smaller cells remain at the center or in the tail of the smear [2] In the work reported here we investigated the distribution of cells in smears that were prepared in the usual way with a wooden spatula used to smear the material directly onto a glass slide To do this repeatedly we developed a model system employing normal exfoliated human cervicovaginal cells and rodent tumor cells suspended in an acellular viscous medium approximating human mucus

Materials and Methods

Collection and Processing of Stock Cell Suspensions

Normal Cervicovaginal Exfoliated Cells Samples were aspirated from the vaginal pool of 20 asymptomatic healthy women who were known to have had normal cytologic smears Each sample was pipetted into 10 ml Tyrode solution (Sigma Chemical Company St Louis Missouri) and centrifuged (110 g) at 5 C for seven minutes The supernatant was decanted and the sediment was thoroughly resuspended in 0 5 ml fresh Tyrode solution to which was added 10 ml of a fixative solution (45 ml absolute methanol and 10 ml water) [3] The suspensions were allowed to stand at 4 C overnight The preparations were centrifuged thereafter at 110 g and the supernatant decanted The

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pellets in the 20 tubes were resuspended in 0 5 ml of fixative and after obtaining a fine-cell suspension with a thin pipette they were pooled together The preparations were stored at 5 C This stock suspension of normal cervicovaginal human cells was used throughout the *in vitro* tests described below

Ascitic Exfoliated Tumor Cells Hyperdiploid-tetraploid Ehrlich ascites tumor cells were taken from two female Balb mice bearing five-day ascites tumors Three milliliters of ascitic fluid was removed from each mouse The cell rich suspensions were added to 10 ml Tyrode solution and centrifuged (110 g) at 4 C for seven minutes The cells were washed twice and the pellets resuspended in 1 ml Tyrode solution which gave a fine suspension free of cell clumps Nine milliliters of fixative was added to each tube and they were stored at 4 C overnight The next day they were centrifuged resuspended in 1 ml fixative and pooled together Eight milliliters of fixative was finally added and the preparation was stored at 4 C This stock suspension of Ehrlich ascites tumor cells (EATC) was used for the tests described below

Recovery of Material from Cell Mixtures

Stock suspensions of EATC and of normal human cervicovaginal cells were mixed so that 10 ml of fixative contained 15% tumor cells and 85% cervicovaginal cells Cell counts were made in a Burker hemocytometer The mixtures were centrifuged (110 g) and the pellets resuspended in 0 5 ml Tyrode solution 1 ml fresh egg white was added and mixed with the cells Tyrode solution was used in order to avoid coagulation of the egg white

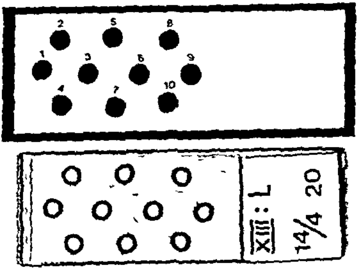

Figure 1
Cardboard template with ten randomly distributed holes of equal size (upper half) used to draw sampling circles on slides containing normal and tumor cells (lower half)

Smearing Technique

Material was collected with a conventional wooden spatula for cervical smears (Ayre spatula Torrent Company Lake Geneva Wisconsin) The smears were done by the same person (Y K) under standardized conditions Smearing was always started on the upper left corner of the slide (the frosted end was on the right side) except when counterclockwise movements were used The motion was similar to that used by gynecologists with the material spread over the entire unfrosted surface of the slide The smears were stained with Papanicolaou stain

Fifty nine smear preparations from the cell mixtures were prepared as follows 17 with clockwise circular motions 14 with counterclockwise circular motions 14 with back-and-forth strokes and 14 with zigzag strokes

Recording of Normal (N) and Tumor (T) Cells

A cardboard template with ten randomly distributed holes of equal size 0 5 cm in diameter (Figure 1) was used to draw sampling circles on each of the 59 slides and the circles were coded The kinds of cells in each circle (normal squamous cells or tumor cells) were recorded

Statistical Methods

To decrease the skewed distribution of the total number of cells (N+T) and of the number of tumor cells (T) in each ring the figures were transformed according to the formulae $(N+T) = {}^{e}\log(N+T+1)$ for the total number of cells and $T = {}^{e}\log(T+1)$ for the number of tumor cells [3] the proportions of tumor cells in each ring were transformed according to the formula $P = \arcsin\sqrt{P}$ where P is the proportion of tumor cells This was done to equalize the comparison of variances in each ring This assumption must be fulfilled before making the analysis of variance The homogeneity of variances was tested with Bartlett s test [1] The means in the results should be interpreted as geometric means since they are transformed data

Results

Distribution of N+T Cells in Randomly Chosen Areas

A total of 11 997 cells (N+T) cells distributed over the ten ringed areas were counted If the cells were randomly distributed 20 3 cells (equal to the arithmetic mean) should have been expected in each circle However the cellular distribution was very skewed (SD = 43 8 with a range of 0 to 641 cells per ring) and the median value (Md = 6 1 cells per ring) differed greatly from the mean

The analysis of variance of the log transformed figures showed a significant difference ($p < 0\ 05$) between the mean values in each ring The number of cells was significantly larger than expected ($p < 0\ 05$) in rings 6 and 9 and was significantly smaller than expected in rings 4 and 10 ($p < 0\ 005$)

The results of the analysis of the transformed data of the four smearing methods are shown in Table I (1) Smears made with clockwise circular motions gave a mean of 7 6 cells per ring There were significant ($p < 0\ 001$) differences in the mean number of cells per ring (transformed figures) The number of cells was larger ($p < 0\ 05$) than expected in ring 6 and smaller in ring 10 (Figure 2) (2) Smears made with counterclockwise circular motions had a mean of 7 2 cells per ring There were significant differences ($p < 0\ 001$) in the mean number of cells per ring (transformed figures) The figures were greater than expected in ring 9 and smaller in rings 4 and 10 (3) Smears with back and forth strokes gave a mean number of 5 4 cells per ring The distribution was very skewed with big differences between the mean numbers of cells per ring The transformed data however showed significant differences between the rings ($p < 0\ 01$) The number of cells was larger than expected in ring 5 and smaller than expected in ring 4 (4) Zigzag strokes yielded the lowest mean value of cells per ring (4 7) There were significant differences between the numbers of cells per ring ($p < 0\ 001$ transformed figures) A significantly larger number of cells than expected was found in rings 6 and 9 fewer cells than expected were found in rings 2 4 and 10

The largest total number of cells (N+T) was found in ring 6 with clockwise motions and the smallest in ring 4 with counterclockwise motions

Distribution of Tumor Cells in Randomly Chosen Areas

A total number of 1 659 tumor cells was found distributed over the 590 areas in the 59 trials In each ring 2 81 tumor cells would be expected with the median value expected to be 0 5 cells per ring In reality however there was a rather uneven distribution of cells in the various areas investigated

The analysis of variance gave a significant difference ($p < 0\ 001$) between the mean number of tumor cells per area (transformed figures) independent of the direction of the movement The greatest number

Table I *Retransformed Data ($e^x - 1$) of the Number of Normal and Tumor Cells Present in Ten Randomly Determined Areas (Rings) in 59 Smear Preparations Made with the Four Traditional Smearing Motions*

Ring code no	Clockwise	Counter clockwise	Back and forth	Zigzag	Total
1	6 5	7 6	3 2	3 7	5 0
2	5 2	7 3	10 7	1 6	5 3
3	12 8	8 5	5 0	7 5	8 3
4	5 3	1 0	1 9	1 2	2 1
5	3 7	14 8	11 7	5 0	8 7
6	28 9	10 9	5 9	10 4	12 5
7	4 8	5 2	3 3	2 7	3 9
8	10 5	14 7	7 0	5 9	9 0
9	15 3	18 9	6 8	23 1	14 7
10	2 4	1 7	2 0	1 7	2 0
$\bar{x}$	7 6	7 2	5 4	4 7	6 1

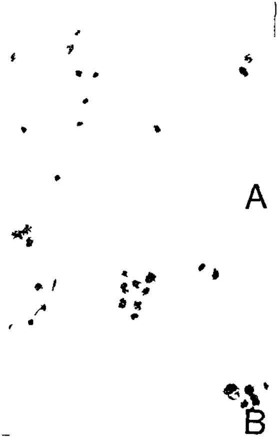

Figure 2
Detail from two areas in the same smear showing normal human squamous cervicovaginal cells and tumor cells (EATC) Note the small number of tumor cells in (A) and the large number of tumor cells in (B) found in rings 5 and 9 respectively Clockwise circular smearing technique (Papanicolaou stain × 100)

of tumor cells was observed in rings 6 and 9 and the lowest in rings 4 and 10 (Table II)

The various smearing methods gave the following results (1) Smears with clockwise circular motions yielded a mean of 1 2 tumor cells per ring The analysis resulted in a significant difference (p < 0 001) between the mean number of tumor cells in the ten rings The highest values were found in rings 6 and 9 and the lowest in rings 5 and 10 (2) Smears with counterclockwise motions gave a mean of 1 3 tumor cells per ring There were significant differences (p < 0 01) in the number of tumor cells contained in the various rings (transformed figures) Numbers greater than expected were found in ring 9 and numbers lower in rings 4 and 10 (3) Back and forth movements yielded a mean of 1 0 tumor cells per ring There were differences in the number of tumor cells between the ten rings (p < 0 01) The numbers found were greater than expected in ring 5 and less in ring 10 (4) Zigzag strokes had the lowest mean number of tumor cells (0 8 cells per ring) The mean numbers of tumor cells in the ten rings differed significantly from each other (p < 0 001) A greater number of tumor cells than expected was found in ring 9 and a lower number than expected in rings 2 and 4

The total number of tumor cells found was higher when using counterclockwise motions than when using clockwise motions The difference was significant The largest number of tumor cells was found in ring 6 when employing a clockwise motion and the lowest in rings 2 and 4 when smears were done with zigzag strokes

Table II *Retransformed Data $(e^{x} - 1)$ of the Number of Tumor Cells Present in Ten Randomly Determined Areas (Rings) in 59 Smear Preparations Made with the Four Traditional Smearing Motions*

Ring code no	Clockwise	Counter clockwise	Back and forth	Zigzag	Total
1	0 8	1 6	1 0	0 5	0 9
2	0 9	1 2	2 3	0 1	1 0
3	1 6	1 2	0 5	1 6	1 2
4	0 6	0 2	0 4	0 1	0 3
5	0 5	2 0	3 7	0 5	1 3
6	4 0	1 8	1 1	1 6	2 1
7	1 1	0 6	0 7	0 4	0 7
8	2 2	2 5	1 5	1 1	1 8
9	2 5	3 2	1 0	3 3	2 4
10	0 5	0 3	0 2	0 3	0 3
$\bar{x}$	1 2	1 3	1 0	0 8	1 1

Proportion of Tumor Cells in Randomly Chosen Areas

The proportion of tumor cells in each ring was transformed before the analysis of variance was performed The analysis of variance then demonstrated significant differences between the proportion of tumor cells when smears were prepared with different motions (p < 0 001) The results are shown in Table III

Significant differences were also found in the proportion of tumor cells found in various rings (p < 0 05) The greatest proportion of tumor cells per ring was found in rings 6 8 and 9 and the smallest proportion in rings 4 and 10

The various smearing motions gave the following results (1) With clockwise circular movements a highly significant proportion of tumor cells was found in ring 8 (p < 0 01) (2) With counterclockwise circular motions a significantly higher proportion of tumor cells per ring (p < 0 01) than expected was found in ring 9 and a smaller proportion than expected in ring 4 (p < 0 01) (3) Back and forth strokes gave significant differences between the various rings (p < 0 01) A higher proportion than expected was found in ring 5 and a smaller proportion in ring 10 (4) Zigzag strokes resulted in significant differences between the proportion of tumor cells found (p < 0 01) the proportions were greater than expected in ring 9 and smaller in ring 2

Table III *Retransformed Data (Sin P)^2 of the Proportion of Tumor Cells Present in Ten Randomly Determined Areas (Rings) in 59 Smear Preparations Made with the Four Traditional Smearing Motions*

Ring code no	Clockwise	Counter clockwise	Back and forth	Zigzag	Total
1	0 040	0 076	0 037	0 013	0 039
2	0 058	0 033	0 076	0 000	0 034
3	0 050	0 085	0 009	0 041	0 042
4	0 020	0 003	0 010	0 004	0 009
5	0 012	0 090	0 111	0 020	0 047
6	0 118	0 077	0 107	0 047	0 086
7	0 051	0 012	0 030	0 011	0 025
8	0 140	0 116	0 037	0 049	0 083
9	0 115	0 129	0 081	0 132	0 114
10	0 014	0 016	0 004	0 011	0 011
$\bar{x}$	0 054	0 054	0 042	0 025	0 043

Discussion

Since tumor cells may be present anywhere on the preparation a cytologic examination for cancer detection requires screening of the entire slide before the absence of tumor cells can be ruled out

In this work some of the factors involved in the distribution of tumor cells on smear preparations when using wooden spatulas were studied Using a known proportion of tumor cells (15%) a similar proportion of tumor cells should be found in randomly distributed areas in the preparation However this was not the case variable proportions of tumor cells were found in defined areas of the slides This uneven distribution appeared not to be entirely adventitious but rather was subject to the motion employed in smearing the material

We used the four most common motions employed by a gynecologist in transferring cells to a slide with a wooden spatula The greatest number of tumor cells was found when smears were made with counterclockwise movements and the lowest with zigzag strokes The causes of this phenomenon are obscure One possibility is that in the zigzag pattern only a portion of the material was spread on the slide because of poor contact between the surface of the instrument and the glass surface Smearing with circular movements may somehow be more thorough than with zigzag strokes Still another possible explanation is that tumor cells that were first attached to the slide were subsequently removed by the overlapping smearing

If this model is an indicator of tumor cell distribution on slides it may be assumed that counterclockwise movements for smearing should be preferable to zigzag strokes However even when using the two less favorable movements of tumor cell yield (back and forth zigzag) some rings have higher proportions of tumor cells than originally used (15%) It is apparent that irrespective of the smearing method increased concentrations of tumor cells can occur in certain areas of the slide Cell concentration was apparent in rings 9 and 6 with ring 9 showing the highest number of tumor cells In contrast a low number of tumor cells was found in rings 4 and 10 Variations in the pressure exerted by the spatula on the surface of the slide during smearing probably play some role in cell distribution Overlapping smearing may also press tumor cells from some areas to other areas that already have tumor cells thus resulting in cell accumulation and may also cause the removal of tumor cells from other areas At this point it should be remembered that tumor cells may remain entangled in the fibers of wooden spatulas[4]

Since the cervical end of the spatula is asymmetrical the angles of the leading and trailing edge of the spreader may affect the cell distribution The hand used by the operator (left or right) to make the smear the differences in cell size and the surface tension may all play roles in the distribution of cells in smears

Whatever the mechanisms it is apparent that tumor cells are distributed to certain areas of the slide with greater frequency by different smearing techniques when using wooden spatulas We are now investigating in more detail the parameters influencing the concentration of tumor cells in certain areas of the slide in hopes of finding ways to detect abnormal cells as directly as possible

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References

1 Kempthorne O The Design and Analysis of Experiments New York John Wiley & Sons 1952 pp 153 158

2 MacGregor RGS Richards W Loh GL The differential leucocyte count J Pathol Bacteriol 51 337 368 1940

3 Ostle B Statistics in Research Ames Iowa Iowa State University Press 1963 pp 338-340

4 Rubio CA The false negative smear II The trapping effect of collecting instruments Obstet Gynecol 49 576-580 1977

5 Rubio CA Thorell B The rapid-flow cytofluorometric identification of tumor cells in exfoliated cell populations Acta Pathol Microbiol Scand [A] 84 443-444 1976

Estrogen Receptor Analysis on Needle Aspirates from Human Mammary Carcinoma

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Solveig Humla T A

Immediately after mastectomy estrogen receptor concentration was measured in fine needle aspirates and in histologic tissue specimens from 13 human breast carcinomas Fine needle aspirates were shown to contain enough material for receptor analysis when the sensitive method of isoelectric focusing in slabs of polyacrylamide gel was used for receptor determination Aspirations with two different needles (outer diameter 0 7 and 1 4 mm) from each tumor yielded highly correlated receptor values ($r = 0\ 97$ $p < 0\ 0001$) There was also a highly significant correlation ($r = 0\ 91$ $p < 0\ 0001$) between the receptor content in needle aspirates and in tissue specimens from the same tumor

The estrogen-binding capacity of human breast carcinoma is widely used to predict the response to endocrine therapy [5] However in many patients with inoperable or recurrent carcinoma surgical specimens for estrogen receptor (ER) determinations are not readily available In most cases material may be obtained from such lesions by means of fine needle aspiration biopsy a method which has been used at Karolinska Hospital since 1955 to diagnose breast tumors [2,3] With this technique 10^6 to 10^7 cells may be obtained Isoelectric focusing in polyacrylamide gel was recently applied to the measurement of estrogen receptors in breast carcinoma [4] It may be performed with only one incubation per tumor analysis and thus a minimal amount of tumor material is needed This feature is essential for the measurement of ER content in fine needle biopsies In this paper the ER levels in aspirates and in histologic specimens are compared

Materials and Methods

Collection of Material

In 13 cases of cytologically diagnosed primary breast carcinoma in women the breast was examined as follows immediately after mastectomy Two separate percutaneous needle aspirates using one needle were collected from the tumor using the technique described by Franzen and Zajicek [2,3] The needle used (outer diameter 0 7 mm) was the same as that routinely used for cytologic diagnosis The collected material from both aspirates was incubated in 0 5 ml of a 5 nM ^{3}H-estradiol solution and the needle was then rinsed with the same solution One drop of the diluted aspirate was smeared onto a slide for microscopic control of the material The same procedure was repeated with a wider needle (outer diameter 1 4 mm) After needle aspirations the breast was incised and three pieces were cut out from different parts of the tumor Each of these pieces was divided into two halves one was used for estrogen receptor analysis and the other was prepared for histologic examination

Microscopic Examination

The cytologic smears were stained with the May Grünwald-Giemsa method The number of tumor cells in the cytologic specimens was graded as none few moderate or numerous The histologic sections were stained with hematoxylin and eosin The type

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of carcinoma but not the grade of malignancy was registered [4]

Biochemical Method

Fine needle aspirates were injected into a constant volume (0 5 ml) of 5 nM ^{3}H-estradiol (141 Ci per mmole New England Nuclear) in ET buffer (10 mM tris HCl pH 7 4 1 5 nM disodium EDTA) and frozen within 15 minutes The material was then kept at −70 C for a few weeks After thawing the aspirates were homogenized in an all glass homogenizer and further incubated at 0 C for 45 minutes The aspirates were then centrifuged at 15 000 g for 20 minutes The supernatants were trypsinized at 10 C for 30 minutes with 5μg of trypsin per optical density unit at 280 nM treated with one third volume of dextran coated charcoal (dextran charcoal 1 10 [w/w]) for ten minutes centrifuged at 800 g for ten minutes and subjected to polyacrylamide-electrofocusing as previously described [8] We used commercially available polyacrylamide gels obtained from LKB Aminkemi Bromma Sweden containing 2% (w/v) ampholine pH range 3 5 to 9 5 and performed the isoelectric focusing for two hours with a Multiphor instrument (LKB) which was cooled with ice water The 200μl samples were placed in the gel near the cathode (pH 8 to 8 5) in sample frames (LKB) Sediments after centrifugation of tumor homogenate contained the cell nuclei and were taken for measurement of DNA according to Burton[1] as a reference for the quantitation of specific binding We recommend that aspirates with more than 10μg DNA be collected for ER assays The ER content in the histologic tissue specimens was measured as previously described [8] Three specimens were analyzed from each tumor and the mean value was calculated by dividing the total number of ER by the total amount of DNA

Statistical Methods

The distribution function of the ^{10}logarithms of ER concentrations from 264 consecutive cases of primary breast carcinoma was shown to be close to the normal distribution The logarithms were therefore used for calculation of the means and for the statistical analyses Concentrations smaller than 0 01 fmol/μg DNA could not be analyzed and were considered 0 01 fmol/μg DNA for the calculations A linear correlation coefficient was calculated between the logarithms of the ER concentrations obtained through the cytologic and histologic techniques The statistical significance of the correlation coefficient r was calculated

Results

The examination of the cytologic control smears revealed a general cytoplasmic lysis caused by the hypotonic ^{3}H-estradiol solution but most of the nuclei were relatively unchanged and had the same appearance as in ordinary cytologic smears The control smears all contained tumor cells and the cellularity of the slides correlated closely with the amount of DNA (Table I) The malignant cell nuclei could be identified readily The admixture of benign epithelial cells stromal cells and lymphoid cells was very slight and negligible

In all cases the diagnosis was confirmed by histologic examination There were two cases of colloid (mucoid) carcinoma all the others were ductal carcinomas The material obtained with fine needle aspiration was sufficient for a reliable determination of ER concentration (Table II) Generally less material was obtained through the finer needle (0 7 mm) than

Table I *Cellularity on Cytologic Slides and Mean Values of DNA Contents in Corresponding ^{3}H Estradiol Solutions*

Number of tumor cells	Number of aspirates	DNA content (μg ± SE)
None	0	0
Few	12	25 2 ± 5 4
Moderate	9	69 2 ± 9 8
Numerous	5	160 2 ± 18 8
Total	26	66 3 ± 11 3

Table II *ER Levels in Needle Aspirates and in Histologic Specimens from Primary Breast Cancers*

	Estradiol bound (fmol/μg DNA)		
Case no	0 7 mm needle	1 4 mm needle	Histologic specimens
1	0 35	0 41	0 97
2	1 11	0 73	0 41
3	0 18	0 54	0 86
4	1 58	2 71	2 72
5	0 01	0 02	0 01
6	0	0	0 01
7	1 08	1 37	4 72
8	0 73	1 34	2 06
9	4 78	2 38	5 37
10	3 85	5 57	2 38
11	1 19	1 74	1 02
12	1 10	1 85	2 33
13	4 47	3 54	1 38

Cases 7 and 9 were mucoid cancers all the others were ductal cancers Cases 2 and 5 were premenopausal all the others were postmenopausal women through the wider one (1 4 mm) The mean value of DNA *contents in the* material obtained with the finer needle was 39 2 ± 10 3 (SE) μg and through the wider needle, 92 1 ± 17 6 (SE) μg The ER concentrations (*Table II*) however were very similar and highly correlated (r = 0 97 p < 0 0001) (*Figure 1*) The ER concentrations obtained with the finer needle were found to have a high correlation (r = 0 91 p < 0 0001) with those obtained in the histologic material (Table II Figure 2) The same high correlation (r = 0 94 p < 0 0001) was found between the ER values (Table II) obtained with the wider needle and with the histologic technique

Discussion

Our data show that estrogen receptor assays may be performed on fine needle aspirates from human breast carcinomas The fine needle biopsy is a quite harmless procedure and no severe complications have been reported in Sweden where the method has been widely employed since 1055 [2,3] In this study a high correlation between the ER values obtained from cytologic and histologic specimens was established Comparison between ER determination on material collected with two different needles indicates a high reproducibility of the method

There are several potential advantages of estrogen receptor assays using needle aspirates as compared to surgical biopsies (1) Aspiration biopsy is essentially a nontraumatic procedure and can readily be em-
ployed in patients with inoperable breast
carcinoma in whom surgical biop diffi
cult and sometimes impossible Th
the collection of material from d
amenable to surgical biop
operative irradiation the
material may be the onl
primary tumor often und
tive changes or totally d
(2) The aspiration proce
and therefore diminishes
and *lymphoid* cells (3) The
be immediately chilled in ic
which reduces receptor

The disadvantages *of the*
needle aspirates are first tha
small for repeated assays with
ever a repeated fine needle biop
easily Second the contamination
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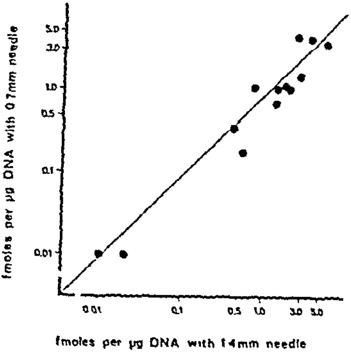
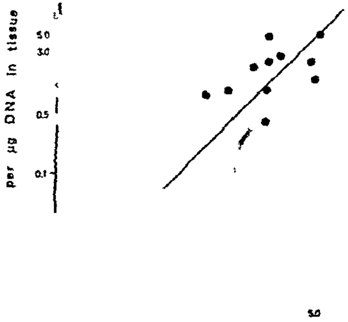


Figure 1
Correlation between ER levels measured in aspirates obtained with 0 7 and 1 4-mm needles Regression line for r = 1 0 is included for comparison



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through the wider one (1.4 mm). The mean value of DNA contents in the material obtained with the finer needle was 39.2 ± 10.3 (SE) μg and through the wider needle 92.1 ± 17.6 (SE) μg. The ER concentrations (Table II) however were very similar and highly correlated ($r = 0.97$ $p < 0.0001$) (Figure 1). The ER concentrations obtained with the finer needle were found to have a high correlation ($r = 0.91$ $p < 0.0001$) with those obtained in the histologic material (Table II Figure 2). The same high correlation ($r = 0.94$ $p < 0.0001$) was found between the ER values (Table II) obtained with the wider needle and with the histologic technique.

Discussion

Our data show that estrogen receptor assays may be performed on fine needle aspirates from human breast carcinomas. The fine needle biopsy is a quite harmless procedure and no severe complications have been reported in Sweden where the method has been widely employed since 1955[2,3]. In this study a high correlation between the ER values obtained from cytologic and histologic specimens was established. Comparison between ER determination on material collected with two different needles indicates a high reproducibility of the method.

There are several potential advantages of estrogen receptor assays using needle aspirates as compared to surgical biopsies. (1) Aspiration biopsy is essentially a nontraumatic procedure and can readily be employed in patients with inoperable or recurrent breast carcinoma in whom surgical biopsies may be difficult and sometimes impossible. The method permits the collection of material from distant metastases not amenable to surgical biopsy. In cases selected for preoperative irradiation the ER analysis on aspiration material may be the only possible method since the primary tumor often undergoes advanced degenerative changes or totally disappears after irradiation[7]. (2) The aspiration procedure selects for tumor cells and therefore diminishes contamination with stromal and lymphoid cells. (3) The aspirated material may be immediately chilled in ice-cold isotope solution which reduces receptor deterioration.

The disadvantages of the material obtained from needle aspirates are first that the amount is too small for repeated assays with other steroids. However a repeated fine needle biopsy can be obtained easily. Second the contamination with serum protein is relatively larger which sometimes results in a peak of sex-hormone binding globulin focusing in the pH region of 5.3 to 5.7 during isoelectric focusing [illegible]

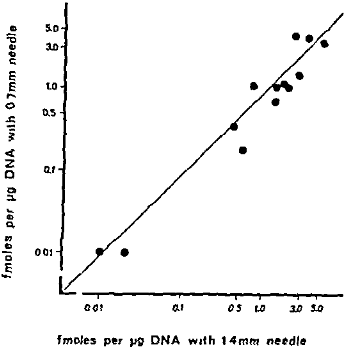

Figure 1
Correlation between ER levels measured in aspirates obtained with 0.7 and 1.4 mm needles. Regression line for r = 1.0 is included for comparison.

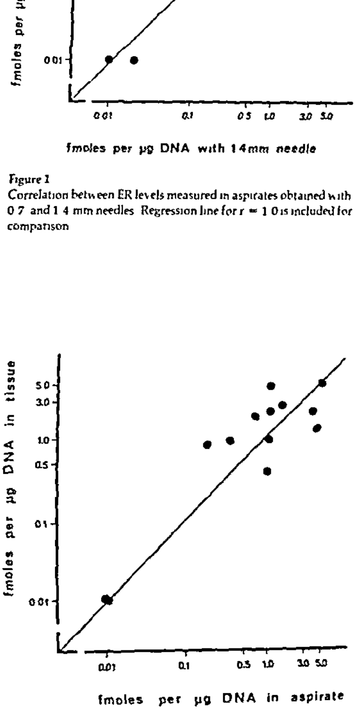

Figure 2
Correlation between ER levels obtained with histologic specimens and with fine needle aspirates (needle 0.7 mm). Regression line for r = 1.0 is included for comparison.

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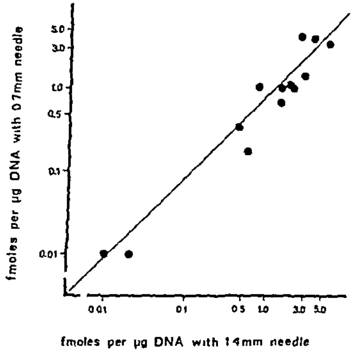

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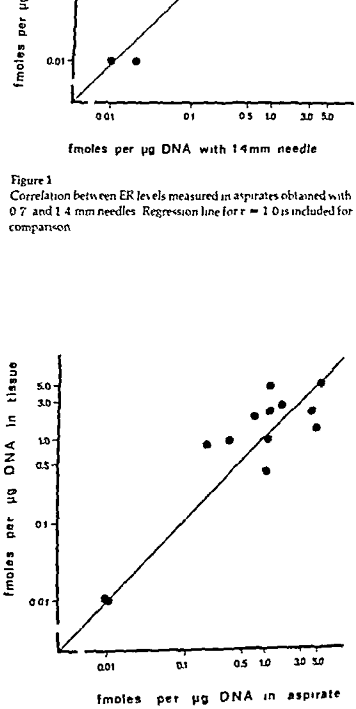

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Abstracts

American Society of Cytology

Scientific Session of the 27th Annual Meeting of the American Society of Cytology San Francisco California October 29-November 3 1979
William W Johnston M D F I A C Program Chairman

Cervical Cytology in Adolescence Is Important

Carole B Boyd, M D
Patricia M Dix M D
Robert J McTaggart C T (ASCP)

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Adolescent women with abnormal cervical cytology smears have been identified as a group at risk for the development of cervical epithelial neoplasia Our patient group serves as a nucleus for the prospective surveillance of cytologic changes in sexually active young women and emphasizes the need for cytologic screening in this age group

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From the Departments of Pathology and of Obstetrics and Gynecology West Virginia University Morgantown West Virginia

Food Contaminants in Sputum

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Coleen Bradford
Ursula Mack
Kathleen Pogacich
Susan Dingler C T (ASCP)

Food contaminants are commonly found in sputum preparations These contaminants usually reside in the oral cavity Our main objective was to identify specific food particles and to compare these particles to cells of known benign and malignant conditions

Thirty two food contaminants were studied Our observations included the following (1) corn was found to resemble keratinizing squamous cell carcinoma (2) apple onion and tomato mimicked the cytologic changes of cytomegaly and (3) cauliflower resembled normal intermediate squamous cells

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0001 5547/80/2401-0058/$02 00 0

Quantitative Assessment of Cell Loss and Cell Dispersal for a Manual Method of Producing a Monolayer of Cervical Cells for Digital Image Processing

Laura J Horn M S
Claire McLatchie C T (ASCP) C M I A C
Elizabeth Stern M D F I A C
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Carolyn A Webber M D

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The temperature recorded in the emergency room was 97 F the pulse was 70 per minute and the respirations 18 The blood pressure recording was 130/70 mm Hg There was no lymphadenopathy Genitalia were described as normal male An intravenous urogram done on a follow up visit to the urology clinic was interpreted as normal urinary tract Cystoscopy disclosed mild inflammation of the trigone and fundus but no stones or tumors Both ureteral orifices had clear efflux Catheterized urine at the time of cystoscopy and each of two subsequent voided specimens examined cytologically contained sheathed microfilariae Distinguishing features of the microfilariae were well demonstrated with the Papanicolaou stain The well-stained nuclei which did not extend into the clear zone and the distinct pale stained sheath led to the positive identification of the microfilariae as *Wuchereria bancrofti*

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From the Cytopathology Laboratory Department of Pathology Kings County Hospital Downstate Medical Center Brooklyn New York

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Neonatal Tracheobronchial Cytology of Bronchopulmonary Dysplasia

Anisa Kanbour M D M I A C
Nalini Doshi M D
Toshio Fujikura M D

A total of 330 tracheobronchial aspirates obtained from 172 neonates were studied in the cytology laboratory at Magee Womens Hospital Of these 162 aspirates from 64 neonates showed the cytologic criteria of bronchopulmonary dysplasia The ages of these infants ranged from six to 200 days Repeated tracheal cellular samplings were performed on 56 babies Sixteen infants died 12 of whom were autopsied

According to the cytologic findings we divided the disease into three states In the early stage the cellular findings included sloughed bronchial cells detached ciliary tufts and metaplastic cells In the intermediate stage the number of metaplastic cells increased and alveolar macrophages and nonciliated columnar cells were observed along with dysplastic cells Occasionally rare and inspissated mucus and Curschmann s spirals were also noted In the late stage two phases were identified progressive and regressive The late progressive phase was characterized by an increase in dysplastic cells alveolar macrophages Curschmann s spirals mucus viscosity metaplastic cells and respiratory casts The late regressive phase showed a decrease in bronchial cells It was further noted that in the late progressive phase evidence of repeated infections was observed along with the cytologic findings mentioned above

The correlation between the clinical and cytopathologic findings of the different stages of bronchopulmonary dysplasia are described

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From the Department of Cytology Magee Womens Hospital University of Pittsburgh Pittsburgh Pennsylvania

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Use of Rat Bladder Tumors as a Model System for Development of New Methods in Urinary Cytology

M Vanderlaan Ph D
Eileen B King M D F I A C
Lois Kromhout C T (ASCP)
P Hara B A
Diana Barrett A B C T (ASCP)
J Walton Ph D

Male Fisher 344 rats were fed 0 05% N-butyl N (4 hydroxybutyl) nitrosamine to induce bladder tumors Pathologic evaluation of animals killed at various intervals was compared with cytologic evaluation of cells dissociated directly from bladders and cells sedimented from overnight urine collections Dissociated cells were prepared by inflating intact bladders with a 0 05% trypsin 5 mM EDTA solution for 15 minutes at room temperature Exfoliated cells were collected into cold culture medium buffered with 50-mM MES (pH 6 0) Unfixed cells may be stored indefinitely without loss of morphology or enzymatic activity if frozen in a medium containing 10% DMSO New diagnostic procedures involving quantitative fluorescent stains for DNA content cell size and the enzymes γ-glutamyltranspeptidase and Cathepsin B were evaluated Cultured rat transitional carcinoma cells (AY 27 cells) were used as positive controls for cytoenzymology Where possible multiple evaluations of the same cells (e g light and electron microscopy or morphology and enzyme activity) were made In this way we established an integrated picture of tumor development and cytology which will permit the testing of new biologic markers to improve the diagnosis of early precancerous lesions

This work was performed under the auspices of the U S Department of Energy by the Lawrence Livermore Laboratory Contract No W 7405 ENG-48 and the National Bladder Cancer Project Grant No CA 23790-01

From the Lawrence Livermore Laboratory Livermore and the University of California Medical Center San Francisco California

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Centrifugal Cytology of Urine

Dorothy Bobbitt C T (ASCP)
Michael Silverman M D
Alan B P Ng M D F I A C
M Bondhus M D
Constantine Railey B S C T (ASCP)
Robert C Leif Ph D

Fixed Papanicolaou-stained dispersions of cells in urine were prepared at a proper concentration for rapid screening by Centrifugal Cytology (Newport Instruments Davie Florida) Samples freshly voided or stored with 10% Transport were concentrated by centrifugation resuspended in Mucolexx (Lerner Laboratories Stamford Connecticut) and pipetted into the three chambers of the new Centrifugal Cytology swinging bucket rotor then centrifuged onto slides and glutaraldehyde fixed with the centrifugal field applied Centrifugal Cytology preparations of 73 known positive samples showed one satisfactory seven (9 6%) bladder and three kidney false negatives eight (11%) suspicious and 54 (74%) positives Of 14 known negatives 11 were found negative and three posttreatment cases contained atypical cells In a comparison study with equal numbers of cells 21 Centrifugal Cytology preparations were positive Cytocentrifuge (Shandon Southern Instruments Sewickley Pennsylvania) preparations showed five (24%) false negatives six (29%) atypical and ten (48%) positives Centrifugal Cytology/Cytocentrifuge mean cell-count ratios for three microscope fields were WBC 8 9 RBC 6 5 squamous 1 82 columnar 3 67 atypical 2 20 and positive 19 5 Transitional and histiocyte mean counts were 10/0 and 16/0 Since Centrifugal Cytology s dispersion area is 14 4 times greater than the Cytocentrifuge s the total recovery ratio is also 14 4 times greater e g 281/1 for positive cells The improved diagnostic accuracy reliability and facility indicate that Centrifugal Cytology is the current technique of choice for urine samples

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From the Papanicolaou Cancer Research Institute and the University of Miami School of Medicine Miami Florida

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Picket Fence Endocervical Cells
What Do They Mean?

Mary Ann Pedigo C T (ASCP)
Linda A Barnes C T (ASCP)
William J Frable M D F I A C

Maternal exposure to diethylstilbestrol (DES) during pregnancy has been implicated as predisposing the adolescent female to clear cell carcinoma of the vagina and cervix Clinically vaginal adenosis mosaicism formation of vaginal hoods etc have been demonstrated with increasing frequency in the DES-exposed young female

During routine screening of cervical Papanicolaou smears and endocervical aspirations of patients attending private and clinic colposcopy units at the Medical College of Virginia many columnar cells of unusual morphology were noted These endocervical cells appeared very elongated and lancet shaped often in tissue fragments with a regimented pattern Their arrangement reminiscent of a wooden picket fence was appropriately labeled as such

During the past 12 months 100 cases of cervical and endocervical smears were collected demonstrating the picket fence endocervical cell A correlation was developed from patient histories particularly DES exposure or other hormonal manipulation colposcopic and histologic findings where available and techniques of cytologic sampling It was anticipated that the picket fence endocervical cell might be a manifestation of DES exposure Results showed that

18% of patients with a well-established history of DES demonstrated these cells However 35% of patients with a history of DES exposure did not demonstrate these cells and 48% of patients with excellent examples of these cells in their smears did not have a history of DES exposure Since other smears from a variety of different clinics and types of patients rarely demonstrate the picket fence endocervical cells further investigation is being carried out to determine the cause of these cells Results will be reported later

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From the Section of Surgical and Cytopathology Medical College of Virginia Virginia Commonwealth University Richmond Virginia

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Fine Needle Aspiration and Endoscopic Brush Cytology

Comparison of Direct Smears and Rinsings

M Jane Smith B S C T (ASCP) C M I A C
Elizabeth Watson C T (ASCP) C M I A C
Sudha R Kini M D

For the past several years cytologic specimens submitted from fine needle aspirations and endoscopy consisted only of direct smears Twenty three percent of these smears were unsatisfactory because of air drying of the cells or the scanty amount of material obtained

To improve the yield and quality of cellular material an additional specimen was prepared by rinsing the brush or needle in a balanced salt solution which was then processed by membrane filtration This combination of direct smears and rinsings decreased our rate of unsatisfactory specimens to almost zero and increased our rate of diagnosis of malignant neoplasms by 29%

In analyzing our data for 1978 the results were as follows Of the 106 malignant neoplasms diagnosed by bronchoscopic cytology or biopsy 21 were diagnosed solely by the needle rinsing Of the 48 malignant neoplasms of the alimentary tract diagnosed by cytology or biopsy 27 were diagnosed solely by the rinsings Last of the 71 malignant neoplasms diagnosed by fine needle aspiration of various sites 18 were diagnosed by the rinsing specimens only

In conclusion for optimum results preparing the two types of specimens from endoscopic brushings and fine needle aspirates is imperative By using this method both the number and quality of the cells available for examination have increased Because of this the number of false negative and unsatisfactory reports has decreased thereby reducing the need for repeat procedures

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From the Department of Pathology Henry Ford Hospital Detroit Michigan

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Morphologic Study of Squamous Carcinoma of the Cervix Uteri in Postmenopausal Women

A Retrospective Study of 50 Cases

Sharon K Rosenthal M D

A computerized retrospective study of the cytologic characteristics of squamous carcinoma of the cervix uteri in postmenopausal women (aged 62 and older) was done using the initial positive cervicovaginal smear from 50 patients with this disease treated at M D Anderson Hospital and Tumor Institute in the period extending from September 1972 through August 1975 A total of 18 nuclear and cytoplasmic parameters which can be evaluated using the light microscope were studied on 100 abnormal cells from each smear The smear background was also examined Relevant clinical data were included on each case All data obtained were computerized for storage and analysis

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From the Department of Anatomical Pathology M D Anderson Hospital and Tumor Institute Houston Texas

evelopment of the Hospital nformation System for ytopathology

race Hasenfang C T (ASCP)
illiam J Frable M D F I A C

n July 1978 the Medical College of Virginia Hospital itiated the first phase of Hospital Information Sys em (HIS) a computerized patient-care system HIS rovides a method of ordering planning and report g the patient-care process and maintains patient illing charges in current status The department of ytology was selected as one of the hospital laborato ies to go on line in this initial phase of implementa ion The purpose of this paper is to describe the sys em of ordering cytology tests and reporting cyto diagnosis that we have developed for use on HIS

The equipment located in each department on the ystem consists of a video matrix terminal (VMT) and a printer The VMT is a modified television combined with a lightpen and a keyboard Information is communicated to HIS through use of the lightpen by selecting words and phrases on the television screen or by typing on the keyboard

Cytology tests are ordered by lightpen selections which carry the user along an ordering pathway that includes selection of specimen type collection procedure information and a patient history matrix On this matrix the user is asked pertinent questions on patient history to which he or she may respond by making appropriate lightpen selections or type ins Test results are communicated to HIS by the cytotechnologist through the accumulation of a series of lightpen selections with some type in modifiers which build into a message containing the complete cytologic diagnosis

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From the Pathology Department Medical College of Virginia Richmond Virginia

Developing Carcinoma (Dysplasia) in the Mammalian Urinary Bladder

William M Murphy M D M I A C
C C Irving Ph D
William N Crabtree B S C T (ASCP)

Bladder cancer often results from a widespread reaction of urothelial cells to carcinogenic stimuli Thus papillary or invasive tumors may coexist with flat premalignant urothelium This abnormal epithelium is thought to resemble dysplasia of the uterine cervix but no report specifically designed to test this hypothesis has appeared in the literature To determine the morphologic features of developing urothelial carcinoma neoplasia was induced in Wistar rats by incorporating the carcinogen BBN in their drinking water Developing lesions were monitored by periodic collections of urine and tissue The specimens were coded and examined by cytology histology and electron microscopy The morphologic changes in the treated bladders were statistically different from those in control tissue They could be divided into three stages The earliest abnormalities were an increase in the number of cell layers a loss of polarity and a slight crowding of nuclei in the tissue sections Among the cellular features there were increased nuclear size and sharply angulated indentations (notches) in the nuclear borders A few nuclei exhibited a finely granular pattern but dusty chromatin predominated In the intermediate stage nuclear crowding became more prominent and there were numerous areas with finely granular regularly distributed chromatin A few nuclei were identified Mitoses were prominent in the tissue sections The early and intermediate stages closely resembled those of mild and moderate dysplasia of the uterine cervix In the late stage as papillary tumors developed the urothelium of the flat areas changed significantly Nuclear crowding and notching remained constant but were overshadowed by the appearance of nucleoli and irregularly distributed chromatin The pattern was finely granular in some cases but coarsely granular in most Nuclear size decreased markedly as the cells became smaller and more numerous Cytologically it was very difficult to distinguish these cells from neoplastic cells emanating from the papillary areas They bore little resemblance to the cells of cervical CIS In this model system the early and inter

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From the Department of Anatomical Pathology M D Anderson Hospital and Tumor Institute Houston Texas

significant SEM may be of value in further studying equivocal specimens even though they were routinely prepared for light microscopic observation
Supported by ASC grant IN112B

From the Departments of Pathology and Microbiology The University of Texas Medical Branch Galveston Texas

The Significance of Cervical Cytologic Dysplasia

Scott W Jordan M D
Nai Lee Smith C T (ASCP)
Lawrence S Dike B S

Cervical squamous dysplasia is associated with a higher risk of subsequent cancer development but there is uncertainty about the relative likelihood of progression and the degree of vigor with which patients having these conditions should be clinically followed

To assess the relative risks associated with the varying degrees of dysplasia we have examined the screening experience of the University of New Mexico cytopathology laboratory for a nine year period during which 182 275 Papanicolaou smears from 96 682 patients were processed The computer accessed patient file was searched for cases with two initial negative smears and having at least five smears in a minimum of five years to allow an adequate time and number of smears for analysis There were 3 390 patients identified having a total of 22 306 smears and a transition analysis was carried out which consisted of comparing each smear to the one after it and tabulating the results Such an analysis allows direct comparison of the relative likelihood that a second smear will be of specified diagnostic category compared to the one preceding it Slight moderate and severe dysplasia were respectively 6 5 20 8 and 68 6 times more likely than was a negative smear to be followed by one showing severe dysplasia or suggestive of carcinoma

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From the Department of Pathology University of New Mexico School of Medicine Albuquerque New Mexico

Diagnostic Pitfalls in Cerebrospinal Fluid Cytology

Misao Takeda M D F I A C
Douglas E King M S C T (ASCP) C M I A C
Hong Yul Choi M D
Kunihide Gomi M D
Warren R Lang M D F I A C

Certain benign cells in cerebrospinal fluid may be a source of erroneous diagnoses and pose diagnostic problems Four such examples are presented cartilagenous cells squamous cells free cells (leptomeningeal cells) and epithelioid cells (histiocytes)

Cartilagenous cells may appear in cerebrospinal fluid as a result of penetration of the intervertebral disc by the needle during lumbar puncture These cells are easily identified by rather distinct cellular characteristics in adults In infants however they may be mistaken for malignant cells because of their immature appearance

Craniopharyngioma may shed mature squamous epithelial cells along with increased numbers of active lymphoid cells in cerebrospinal fluids If the squamous epithelial cells are mature they may be interpreted as contaminants from the skin puncture If immature they may be difficult to differentiate from a well-differentiated squamous cell carcinoma

In cases of cerebral infarction the proliferating meningeal lining may shed large mononuclear cells with prominent nucleoli Mitoses are frequent These cells may be erroneously diagnosed as malignant

On rare occasions chronic granulomatous lesions such as sarcoidosis may involve the brain and shed many active histiocytic cells into cerebrospinal fluids These active cells may easily be confused with reticulum cell sarcoma

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From the Departments of Pathology and of Cytotechnology Jefferson Medical College and College of Allied Health Sciences Thomas Jefferson University Philadelphia Pennsylvania mediate changes of developing urothelial carcinoma are similar to each other and to those described for mild and moderate dysplasia of the uterine cervix Advanced lesions have many of the cellular features of malignancy A direct counterpart to CIS of the uterine cervix cannot be distinguished

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From the Departments of Pathology and Urology University of Tennessee Center for the Health Sciences Memphis Tennessee

ments calcifications and in fresh wet mounted preparations cholesterol crystals

Pituitary adenomas rarely exfoliate and are diagnosed cytologically by aspiration In our cases aspiration through craniotomy yielded uniform sinusoidal or papillary epithelial material diagnostic of chromophobe adenomas

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From the Department of Pathology University of Texas Medical Branch Galveston Texas

Cytology of Common Primary Midline Brain Tumors

Paul Zaharopoulos M D M I A C
Jick Yee Wong M S C T (ASCP) M T (ASCP)

A variety of primary midline brain tumors occupying the pineal pituitary axis can appear under similar clinical settings sharing common symptoms by obstructing the ventricular system and compressing vital midline structures Improved radiodiagnostic studies can delineate them and cytology both of exfoliated and cerebrospinal fluid and of aspiration specimens can differentiate them

Included in the 11 such lesions we encountered were three germinomas (atypical midline teratomas) five craniopharyngiomas and two chromophobe pituitary adenomas which are the most frequently occurring types

Germinomas histologically resemble seminomas often with other teratomatous elements (as in one of our cases) They readily exfoliate and in this series cerebrospinal fluid obtained by lumbar puncture yielded relatively large epithelium like or true epithelial cells with immature nuclear characteristics which included prominent nucleoli These under a different setting could have suggested a metastatic large-cell type carcinoma

Craniopharyngiomas become cystic and cytologically are diagnosed usually by aspiration methods Aspirations during craniotomy or ventricular shunt procedures in our cases yielded mature keratinized squamous elements reactive granulomatous ele-

Scanning Electron Microscopy (SEM) of Alcohol-Fixed Specimens

Steven N Becker M D
Jick Yee Wong C T (ASCP)
Alan A Marchiondo M S
C P Davis Ph D

Specimens evaluated cytologically by light microscopy are routinely fixed in alcohol whereas specimens studied by scanning electron microscopy (SEM) are often carefully cleansed and fixed with glutaraldehyde In the latter studies mature squamous cells have microridges a variety of malignant cells have microvilli and other benign and malignant cells have a ruffled topography Our study was made to determine if these features are also appreciable in cytologic specimens fixed in alcohol and stained by the Papanicolaou method for routine diagnostic evaluation

In normal bronchial washings ciliated columnar cells were obvious and microridges were detected in several squamous cells Microvilli although sometimes coarse and blunted were present in adenocarcinoma squamous carcinoma histiocytic lymphoma and malignant mesothelioma cells in fluid specimens Neuroblastoma cells in a smear of bone marrow aspirate and lymphocytic lymphoma cells in spinal fluid had ruffled surfaces

Compared to glutaraldehyde fixed preparations our studies were not nearly as elegant However should topologic features prove to be diagnostically

significant SEM may be of value in further studying equivocal specimens even though they were routinely prepared for light microscopic observation
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Rapid Staining Methods Employed in Fine Needle Aspiration of the Lung

Hyun Y Pak M D
Susan Yokota C T (ASCP) C M I A C
Raymond L Teplitz M D

Fine needle aspiration for cytologic diagnosis has gained popularity and has been successfully employed as a diagnostic technique With respect to the lung sputum and bronchoscopic cytology often does not yield diagnostic material from peripheral lesions even where the lesion is visible on radiographs Needle aspiration under fluoroscopic guides is a method which facilitates procurement of diagnostic material and diagnostic accuracy

Quick staining of aspiration material for rapid diagnosis as employed in our institution enables the aspirator to determine the adequacy of the specimen before releasing the patient thereby ensuring higher diagnostic accuracy The quick-staining procedure takes from three to five minutes and is evaluated immediately by the cytopathologist If adequate material is not obtained with the first aspiration subsequent aspirations are performed unless the patient s clinical condition precludes continuation

Recently a review of 32 consecutive cases of needle aspiration of the lung was performed A majority of these cases involved peripheral lesions ranging in size from 1 1 to 11 cm in the greatest diameter In 17 of these cases quick staining for a rapid diagnosis was employed Ninety four percent diagnostic accuracy was achieved in these 17 cases whereas 80% accuracy was achieved in those cases in which rapid stains were not employed

We conclude that lung needle aspiration cytology is of great diagnostic value with high accuracy in the diagnosis of peripheral pulmonary lesions Rapid staining used as a monitoring guide for the adequacy of aspiration material also significantly contributes to improved diagnosis

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From the Division of Cytogenetics and Cytology City of Hope National Medical Center Duarte California

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Changing Patterns of Cervical Disease in a Student Population

Jeannette Strumwasser C T (ASCP)
Dorothy L Rosenthal M D M I A C
Edward Wiesmeier M D

An apparent fivefold increase in disease of the uterine cervix in young women was explored The Cytology Service at the UCLA Center for the Health Sciences has examined some 4 000 Papanicolaou smears from the UCLA Student Health Service over the past five years (1973 1978) Originally apparently free of cervicovaginal disease these women now have significant inflammatory atypia dysplasia and even carcinoma *in situ* Reasons for this increase in a supposedly stable population were investigated and plans for a prospective study were outlined

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From the Department of Pathology UCLA School of Medicine and the Student Health Service and the Cytology Service UCLA Center for Health Sciences Los Angeles California

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Use of Blood Group Isoantigens in Distinguishing Benign and Malignant Cells in Effusions

Nancy J Smith C T (ASCP)
Bruce R Dziura M D
Bernard Gondos M D

The work of previous investigators has shown the presence of A B and H blood group isoantigens on the surfaces of cells from a variety of normal tissues as well as their progressive loss during the development of dysplasia carcinoma *in situ* invasive carcinoma and metastatic carcinoma This study employed an indirect immunoperoxidase technique to study blood group isoantigens on the surfaces of cells in effusions to determine the usefulness of the procedure in distinguishing benign and malignant speci mens After first establishing with the use of histologic material that mesothelial cells normally possess blood group isoantigens studies were performed on a series of benign and malignant effusions Both normal and atypical mesothelial cells stained positively for isoantigens while malignant cells from a variety of tumor types failed to take the stain The significance of the findings and the limitations of the procedure are discussed with reference to its application in routine cytologic diagnosis

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From the Department of Pathology University of Connecticut Health Center Farmington Connecticut

Cytomorphology of Ganglioglioma of the Central Nervous System

Adolfo Firpo M D
Carolyn W Watson M D

Nine cases of ganglioglioma of the central nervous system (CNS) were reviewed Three patients had cerebrospinal fluid (CSF) specimens submitted for cytologic examination prior to surgery One of them had abnormal cells suggestive of a primary CNS tumor Touch preparations of two tumors were made at the time of surgery They contained numerous neurons glial cells connective tissue and reactive vascular elements Neuronal and glial components of the tumor were further characterized in the tissue sections with NISSL Bodian and PTAH stains

Cells similar to the abnormal ones in the CSF were sought in the touch preparations and tissue sections The nuclear size of morphologically comparable cells was determined in the different preparations and correlated with the cell types This method permitted the identification of nervous tissue components in the CSF Some cells were suggestive of abnormal neurons and other cells appeared to be of glial origin These findings suggest that if this tumor reaches the ventricular system or the subarachnoid space it may exfoliate intrinsic cells which can be identified in the CSF sample This report also illustrates the applicability of simple quantitative determinations in the identification of exfoliated cells of uncertain origin

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From the Papanicolaou Cytology Laboratory New York Hospital Cornell Medical Center New York New York

Fine Needle Aspiration Cytopathology of Bronchial Carcinoid Tumors
An Analytic Study of the Cells

Deborah Cafer B S C T (ASCP) C M I A C
Thomas A Bonfiglio M D F I A C
Stanley F Patten Jr M D Ph D F I A C

While frequently considered to originate from a common stem-cell of neuroendocrine origin bronchial carcinoid tumors and small cell anaplastic carcinomas differ significantly in their biologic potential and treatment Patient management is often dependent on the diagnostic specificity of a pulmonary fine needle aspiration specimen

This study evaluated the cellular features in fine needle aspiration samples from seven bronchial carcinoid tumors and four small cell anaplastic carcinomas of intermediate type Planimetric measurements were performed on tracings of approximately 2 000 cells A semiquantitative analysis of specific cytoplasmic and nuclear features was also obtained

Significant quantitative and qualitative differences in the cytomorphology of cells derived from bronchial carcinoid and small cell anaplastic neoplasms were obtained clearly demonstrating that differentiation of these neoplasms is possible on a cytopathologic basis in fine needle aspiration samples The differential diagnostic features identified in this study are discussed

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Thomas A Bonfiglio M D F I A C
Stanley F Patten Jr M D Ph D F I A C

While frequently considered to originate from a common stem-cell of neuroendocrine origin bronchial carcinoid tumors and small cell anaplastic carcinomas differ significantly in their biologic potential and treatment Patient management is often dependent on the diagnostic specificity of a pulmonary fine needle aspiration specimen

This study evaluated the cellular features in fine needle aspiration samples from seven bronchial carcinoid tumors and four small cell anaplastic carcinomas of intermediate type Planimetric measurements were performed on tracings of approximately 2 000 cells A semiquantitative analysis of specific cytoplasmic and nuclear features was also obtained

Significant quantitative and qualitative differences in the cytomorphology of cells derived from bronchial carcinoid and small cell anaplastic neoplasms were obtained clearly demonstrating that differentiation of these neoplasms is possible on a cytopathologic basis in fine needle aspiration samples The differential diagnostic features identified in this study are discussed

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From the Cytopathology Division Department of Pathology University of Rochester Medical Center Rochester New York

Cytopathology of Pleomorphic Liposarcomas in Pleural Fluid

Kim R Geisinger M D
Bernard Naylor M D F I A C
Theodore F Beals M D
Patricia M Novak B A C T (ASCP)

Although they are rare liposarcomas are among the more common malignant neoplasms of soft tissues Descriptions of their cells in serous fluids are even more rare We shall describe liposarcoma cells in pleural fluids from two patients with typical pleomorphic liposarcomas

The fluids contained cells readily recognized as malignant They were extremely large and pleomorphic with diameters ranging from 170μm to 370μm Almost all of the neoplastic cells were solitary They were generally mononuclear with eccentric nuclei that were round oval or irregularly polypoid Usually the chromatin occurred as large irregularly shaped clumps In one case macronucleoli were prominent Many cells possessed cytoplasmic vacuoles which varied considerably in size and number The indistinct cellular borders blended into the smear background

With transmission electron microscopy the liposarcoma cells were polygonal or elongated Nuclear shapes varied from round to deeply indented Unorganized rough endoplasmic reticulum was abundant Microfilaments were focally prominent Most cells demonstrated cytoplasmic lipidic droplets Cellular borders were highly irregular with long thin processes With scanning electron microscopy the surfaces were remarkably pleomorphic with ruffles blebs and microvilli

The major diagnostic feature of liposarcomas which is perceivable by light microscopy is the presence of solitary pleomorphic giant cells with cytoplasmic vacuoles

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From the University of Michigan and Veterans Administration Medical Center Ann Arbor Michigan

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The Cytologic Diagnosis of Blastomycosis in Pleural Effusions

Jamie L Covell C T (ASCP)
Edwin H Lowry Jr C T (ASCP)
Philip S Feldman M D

Until recently pleural involvement in blastomycosis was considered uncommon however recent studies have shown pleural reactions in a significant number of cases The applicability of cytology in diagnosing blastomycosis has been established in respiratory material but there are no known reports of this diagnostic method being used in pleural effusions The two cases presented here demonstrate the primary role cytology can play in the diagnosis of pleural involvement of blastomycosis In one case of acute blastomycosis the recognition of the diagnostic budding yeast forms in cytologic preparations of pleural fluid was the first indication of the true nature of the patient s illness for sputum culture had failed to yield diagnostic results The second case of chronic blastomycosis had proven organisms on sputum culture but cytologic evaluation of the pleural fluid was the primary indication of pleural involvement This was confirmed by pleural biopsy but fluid culture was negative Considering the time interval required for blastomyces culture and the unreliability of skin and serologic tests cytologic examination of pleural fluid can give accurate and more rapid results This is of particular significance for pleural effusions of undetermined etiology in endemic areas

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From the Cytology Laboratory Department of Pathology University of Virginia Medical School Charlottesville Virginia and the Cytology Laboratory Department of Pathology Medical College of Virginia Richmond Virginia

omparison Between Diagnostic esults Obtained by Fine Needle spiration Cytology Versus True Cut TC) Open Biopsies

onald J Innes Jr M D
hilip S Feldman M D

even cases are presented in which fine needle aspira ion (FNA) cytology provided the definitive diagno is in cases with negative tissue biopsies obtained by TC (three cases) and open biopsies (four cases) Included are a breast carcinoma two metastatic liver lesions two neck lesions a groin lymph node and a retroperitoneal tumor FNA permits a better sampling of the tumor since it is easier to take several specimens from different points than with the TC biopsy This was exemplified by the breast case where a TC biopsy was negative and subsequent FNA was diagnostic of carcinoma Mastectomy confirmed the diagnosis TC biopsies in two cases with questionable liver metastases were negative Ultrasound-directed FNA in both confirmed the presence of metastases The retroperitoneal mass was initially approached by exploratory laparotomy with multiple biopsies however no tumor was diagnosed Subsequent ultrasound-directed FNA demonstrated a malignant tumor possibly seminoma Examination of the scrotum and orchiectomy cytology on lymph nodes in three cases were diagnostic of malignancy Subsequent open biopsies showed no tumor in the initial sections Because of the FNA diagnosis additional serial sections confirmed the presence of carcinoma This series demonstrates that FNA cytology should be considered more often as the initial diagnostic procedure

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From the Department of Pathology University of Virginia Medical School Charlottesville Virginia

The Value of Ethanol as a Preservative in Urinary Cytology

William N Crabtree B S C T (ASCP)
William M Murphy M D M I A C

Although 50% ethanol is widely used as a preservative in urinary cytology the value of this procedure has received little systematic analysis The purpose of this study was to examine the preservative qualities of 50% ethanol when added to freshly collected urine for both immediate and delayed processing All urinary specimens of sufficient volume were thoroughly mixed and divided into four equal 10 ml aliquots They were processed as follows (1) The unaltered specimen was processed within four hours of collection (2) Urine was mixed with an equal volume of 50% ethanol refrigerated for 30 minutes and processed (3) The specimen was centrifuged reconstituted with 0 9% saline stored for 24 hours and processed (4) The centrifuged specimen was reconstituted with 25% ethanol in saline stored for 24 hours and processed Millipore filters were prepared for each part and stained with a modified Papanicolaou technique Slides were coded and evaluated for total cell number aggregation cytoplasmic and nuclear preservation and characteristics of the background Only intermediate and basal urothelial cells were evaluated The results indicated that (1) degenerative changes occurring *in vivo* are common among urothelial cells and probably represent the single most important factor in cell preservation in voided urine (2) with few exceptions fresh unfixed urine yields the greatest number of well preserved cells (3) centrifugation with reconstitution in either saline or 25% ethanol/saline and 24 hour storage results in increased cellular degeneration For Millipore filter preparations addition of 50% ethanol has no apparent advantage even when processing must be delayed for several hours

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From the University of Tennessee Center for the Health Sciences Memphis Tennessee

Megakaryocytes in Pleural and Peritoneal Fluids

Prevalence Significance Morphology and Cytohistologic Correlation

Neelman Kumar M D
Bernard Naylor M D F I A C

Megakaryocytes are rarely found in serous fluids We examined 4 844 serous fluids from 3 279 patients and found megakaryocytes in pleural and peritoneal fluids from five of these patients The diagnoses in these patients were agnogenic myeloid metaplasia (three) chronic granulocytic leukemia (one) and lymphocytic lymphoma (one) All of these patients had persistent serous effusions Pleural and peritoneal nodules of extramedullary hematopoiesis were found at necropsy in the two patients with agnogenic myeloid metaplasia

The presence of megakaryocytes in a serous fluid signifies the development of extramedullary hematopoiesis which may be a compensatory change (myeloid metaplasia) due to damage to the bone marrow or a neoplastic change (agnogenic myeloid metaplasia)

Normal mature megakaryocytes are large cells with polymorphous nuclei Immature and abnormal megakaryocytes display anisocytosis and aberrant nuclear forms They may be confused with giant carcinoma cells sarcoma cells and Reed Sternberg cells They may also resemble benign giant mesothelial cells and histiocytes Because agnogenic myeloid metaplasia may manifest itself by tumor formation it is important not to mistake megakaryocytes for cells of carcinoma or sarcoma

We illustrated megakaryocytes in serous fluids and compared them with a variety of neoplastic and non-neoplastic cells Cytohistologic correlation was demonstrated in the two cases where necropsy was performed

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From the Department of Pathology University of Michigan Ann Arbor Michigan

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Statistical Comparison of Aerosol-Induced and Spontaneous Sputum Specimens in the Tyler Asbestos Workers Program

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S Donald Greenberg M D
George A Hurst M D
Charles G Spivey M D
Margaret Farley C T (ASCP)
Linda C Mabry C T (ASCP)

Cytopathologic diagnoses made from aerosol induced and spontaneous cough specimens from the same individual may vary More than 10 000 sputum specimens induced and spontaneous have been processed in a surveillance program involving semi annual clinical and laboratory examinations of 856 former asbestos workers and 187 controls At each clinic visit sputum was induced by transoral inhalation of an aerosolized solution of 8% sodium chloride Subsequently a pooled three-day collection of spontaneous early morning cough samples was also mailed to the laboratory During the first two years of the program slides were prepared by the pick and smear method later the Saccomanno technique was used The degree of cellular atypia and the number or absence of ferruginous bodies were noted in each specimen

Statistical analysis of the data revealed that ferruginous bodies found in the spontaneous sputum specimens showed a higher correlation with chest X rays and pulmonary function tests than did ferruginous bodies from aerosol induced specimens Consequently a study was undertaken to compare the results of aerosol induced and spontaneous cough specimens from the same individual Significantly higher scores (that is more atypical samples) were obtained from the spontaneous specimens Statistical methods used in the analysis included the paired-sample t test and the Wilcoxan matched pairs signed rank test The difficulty of obtaining spontaneous specimens from nonsmokers was also considered in the analysis

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From the University of Texas Health Center in Tyler Tyler Texas and the Baylor College of Medicine Houston Texas

Evaluation of Collection and Preservation Techniques for Urinary Cytology

John C Pearson M D
Lois Kromhout C T (ASCP)
Eileen B King M D F I A C

Urinary cytology remains of controversial value despite 25 years of clinical use Degeneration of cells during the time between collection and evaluation is a cause of difficulty in interpreting the cellular findings Also it appears that accuracy varies widely between different institutions partly because each has its own protocol for collection preservation and preparation of samples

In an effort to decrease cellular degeneration and promote standardization of methods we attempted to define the optimal conditions for urinary cytology In separate experiments under controlled conditions freshly dissociated human and rat bladder cells both benign and malignant were collected and suspended in urine at pH 4 5 6 0 and 8 0 One half of each suspension was added to an equal volume of methanol and aliquots were then examined at 0 2 24 and 72 hours for preservation of nuclear and cytoplasmic morphology and staining reactions Cytologic detail seems to be better preserved at pH 4 5 with little difference between fixed and unfixed specimens At higher pH cellular morphology rapidly and progressively deteriorates Perhaps urine more alkaline than 4 5 should be acidified when collected before storage or preparation for cytologic study The cellular morphology of specimens stored more than two hours can be improved by the addition of 50% methanol

This work was performed under the auspices of the National Cancer Institute Grant R26CA23790 and the U S Department of Energy Contract NIHCA13556

From the Department of Urology and the Division of Cytology University of California School of Medicine San Francisco California

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Two Histologic Types of Early Gastric Carcinoma and Their Cytologic Presentation

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Kazuya Tamura M D
Shigeo Ohoki M D
Minoru Fujimoto M D
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One hundred nineteen cases of early gastric carcinoma were studied as to age sex histologic cell types depth of penetration of the cancer metastasis to the regional lymph nodes and cellular changes of the mucosa near the foci of cancer Two distinctly different cellular types were identified One was the intestinal type and the other was a diffuse mixed-cell type

The intestinal type occurred in the older age group while the diffuse mixed-cell type was noted in the younger age group of both sexes Once cancer penetrated through the muscularis mucosae there was an increased incidence of metastasis to the regional lymph nodes regardless of the cellular type

In contrast to this only one diffuse mixed-cell type which was limited to the mucosa and none of the intestinal type of intramucosal cancer had metastasis to the regional lymph nodes On these histologic bases cytologic characteristics of the two types of gastric carcinoma were summarized

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From the Jefferson Medical College Thomas Jefferson University Philadelphia Pennsylvania the Department of Gastroenterology Osaka National Hospital Osaka Japan and the Clinical Laboratory Yokohama City University Hospital Yokohama Japan

Cytologic Evaluation of Ocular Lesions

Tamara L Sanderson M D , M S P H
Wilhelmina Pustai C T (ASCP)
Henry Gelender M D

A retrospective study was undertaken to evaluate the diagnostic accuracy and establish the cytomorphologic criteria for normal ocular specimens and for benign and malignant ocular *lesions It included* 84 cytologic specimens from 59 patients with clinically *suspicious* lesions of which 72 were conjunctival scrapes ten were vitreous fluid aspirates one *was a* corneal scrape and one was an iris cyst aspirate Tissue correlation was *available for* 27 patients A control group of 20 normal conjunctival scrapes was also evaluated

Cytomorphologic criteria for normal ocular specimens inflammatory lesions and premalignant and malignant *lesions are presented These lesions* included dysplasia (seven cases) carcinoma *in situ (four cases)* squamous *cell* carcinoma (five cases) Meibomian gland carcinoma (*three cases*) basal cell carcinoma (two cases) metastatic adenocarcinoma (one case) melanoma (two cases) *and neuroblastoma* (one case) The remaining lesions were nonspecific *inflammatory* changes and normal variants The diagnostic accuracy of ocular cytology is discussed The results of this study indicate that cytologic examination of ocular specimens can be a valuable adjunct in the workup of suspected neoplastic eye *lesions*

From the Section of Cytology and Bascom Palmer Eye Institute University of Miami Miami Florida

Value of the Maturation Index as an Indicator of the Serum Estrogen Concentration During Treatment with Gonadotropins

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Barbara J Kaplan C T (ASCP) C M I A C
Charles M March M D
Richard P Marrs M D

Smears were taken each day from the middle third of the lateral vaginal wall of 14 infertile women who were being treated *with daily intramuscular injections* of human menopausal gonadotropins (HMG) *to induce ovulation* Each smear was stained with modified Papanicolaou stain and screened for the maturation index (MI)

In 23 HMG treatment cycles MI *generated from* the vaginal smears was compared to daily serum estrogen concentrations In seven of the 23 treatment cycles evaluated the MI predicted a rise in serum estrogen However in 16 of the 23 cycles the MI did not predict the increasing serum estrogen concentrations

It was concluded that *the vaginal epithelium may* not respond or may respond too slowly in relation to the rapidly rising serum estrogen concentrations and therefore does not accurately predict the dynamic changes that occur with HMG therapy Therefore although inexpensive easily obtained and rapidly performed the cytologic examination of the vaginal *epithelium cannot be used to predict* serum estrogen concentrations during HMG treatment

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From the Departments of Surgical Pathology and of Obstetrics and Gynecology Los Angeles County University of Southern California Medical Center and the University of Southern California *School of Medicine* Los Angeles California

Cerebrospinal Fluid Cytology in Five Patients with Cerebral Cysticercosis

R R Wilber M D
Eileen B King M D F I A C
E L Howes Jr M D

Cerebral cysticercosis (taenia solium) is common in Central American countries but it is relatively infrequent in the United States We have had an opportunity to study five documented cases of cerebral cysticercosis This report will emphasize distinctive cellular findings in cerebrospinal fluid (CSF) and the pertinent clinical features of these cases All five patients were born in Central America and spent most of their lives in these countries History of previous parasitic infection was variable Neurologic symptoms and signs were usually nonfocal The routine examination of CSF revealed a wide range of elevated leukocyte counts in all five cases however eosinophilia was identified in only two The cytologic examination of CSF was distinguished by the presence of a marked pleocytosis In three of these cases the variability and atypia were extreme leading to a consideration of a CNS lymphoma Eosinophiles were not observed in the cytologic preparations from these cases No structures diagnostic of the etiologic agent (such as hooklets) were observed We conclude that the clinical findings coupled with an awareness of the cellular pleomorphism in the CSF should suggest an inflammatory lesion rather than a lymphoma However a diagnosis of cysticercosis can only be considered as a differential possibility if specific evidence of the etiologic agent is obtained

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From the Department of Pathology University of California and the Department of Pathology San Francisco General Hospital San Francisco California

Intrathecal Chemotherapy
The Cytopathologist s Dilemma

W B Dupree M D
M M Sullivan M B B Ch
J Gilmore M T C T (ASCP)

With the advent of chemotherapy protocols for leukemia and lymphoma using decision branch points based on the presence of blast cells in the cerebrospinal fluid the cytopathologist now assumes a decisive role in the management of these patients A series of patients with leukemia was followed through courses of intrathecal chemotherapy and the CSF was examined using the Millipore filter technique The hematopoietic cells using this technique have different characteristics from those examined using other methods Therefore it becomes necessary to have definite criteria for the morphologic classification of these cells Guidelines are proposed for the identification of blasts and other cells in the CSF In addition the effects of chemotherapy on cellular morphology are described

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From the Department of Pathology Dartmouth Hitchcock Medical Center Hanover New Hampshire

Cytopathology of Pericardial Effusion

Hossein M Yazdi M D
Steven I Hajdu M D F I A C
Myron R Melamed M D F I A C

During a period of four years 158 cytologic specimens obtained from pericardial effusions of 120 patients were examined Malignant tumor cells were identified in 90 (57%) specimens from 72 (60%) patients Eighty percent of the tumors were epithelial and 20% were nonepithelial in origin The most com

mon epithelial tumors were pulmonary (56%) and mammary (39%) neoplasms and most were adenocarcinomas In general the cytologic smears were moderately cellular with blood and occasional atypical mesothelial cells in the background Reactive cells such as histiocytes and lymphocytes were rarely seen in cytologically positive specimens

Pleomorphism of nuclei and prominence of nucleoli were dominant features of pulmonary adenocarcinomas Tumor cells of mammary carcinoma were most uniform and often formed cell balls Epidermoid carcinoma was characteristically poorly differentiated having a striking resemblance to adenocarcinoma In most instances several months elapsed between the initial diagnosis of the primary neoplasm and positive pericardial cytologic findings a majority of the patients died within ten months after malignant tumor cells were recovered from the pericardial effusion These and other pertinent clinicopathologic findings are presented and the cytomorphology of various malignant neoplasms is illustrated

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From the Cytology Service Department of Pathology Memorial Sloan Kettering Cancer Center New York New York

Immunoperoxidase Staining of Trichomonas vaginalis *in Cytologic Material*

Caroline M O Hara M D C T (ASCP)
C M I A C
W A Gardner Jr M D
B D Bennett M D

In recent years the literature has been inundated with reports of applications of immunocytochemical techniques to histopathology The remarkable popularity of these techniques relates to several factors (1) specific localization and identification of antigenic sites (2) use of tissue which has been fixed and processed by routine procedures and (3) applicability to a wide variety of tissues and antigens Although interest in this procedure as a diagnostic tool in histopathology has been expanding exponentially we are not aware of the application of these techniques to diagnostic cytology

Trichomonas vaginalis is a common pathogen of the female genitourinary tract and can usually be identified with ease in routine cytologic preparations of gynecologic material However the unequivocal identification of these organisms in specimens from unusual i e extragenital sites may be extremely difficult In the course of studies on the histopathology and pathogenesis of trichomonal infections specific antisera to *T vaginalis* were produced Using these antisera conventional immunocytochemical techniques have been modified and applied to a variety of cytologic specimens With this methodology trichomonal organisms have been identified in specimens from both usual and unexpected locations These findings plus the potential applications of similar methodologies to other areas of cytopathology form the basis of this paper

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From the Laboratory Service Veterans Administration Medical Center and the Department of Pathology Vanderbilt University School of Medicine Nashville Tennessee

The Cytopathologic Diagnosis of Primary Liver Carcinoma

Linda M Shrader A B C T (ASCP)
Charles N Carney M D

Study of cytologic material of the liver obtained by needle has been established as a useful diagnostic tool The advantages are that (1) 20% to 30% of tumors will be detected in the cytologic specimen alone in contrast to the surgical specimen (2) a diagnosis is available in a very short time and (3) detailed cytologic study supplements the tissue study for greater accuracy in tumor typing

Approximately 400 liver aspirates have been studied for diagnosis at North Carolina Memorial

Hospital since 1970 Of them 17 cases were primary hepatic carcinomas Specimens were obtained in conjunction with needle biopsy tissue and by fine needle aspiration The cases included well and poorly differentiated hepatocellular carcinomas cholangiocarcinomas mixed hepatocellular and cholangiocarcinomas and hepatoblastoma All cases have been followed up with biopsy autopsy ultrastructural studies or clinical course Cases of hepatic carcinoma diagnosed cytologically from metastatic sites also were studied The cytomorphologic spectrum of normal reactive and malignant hepatic cells is presented and discussed

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From the Cytopathology Laboratory Department of Pathology University of North Carolina School of Medicine Chapel Hill North Carolina

Direct Endometrial Aspiration Cytology
An Evaluation

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Samona Willie C T (ASCP) C M I A C
Karen J Cove M D M I A C

Early detection of uterine corpus malignancies especially in asymptomatic women remains an elusive goal Although our previous studies on cervical endocervical canal aspiration cytology were fruitful the technique did not prove sensitive or specific enough for us to advocate this method for mass screening of women at risk The development of the Curity Isaacs* endometrial aspirator is an attempt to improve the diagnostic yield for intrauterine lesions This painless disposable device permits the direct sampling of endometrial epithelium without contamination of the cell sample by material from the endocervical canal or vaginal pool and without the artifact of desquamated degenerated cells

We have studied cell samples obtained by this simple outpatient technique from 1 000 perimenopausal and postmenopausal women in our prepaid health care program The results of cytodiagnostic sampling are reported correlated with appropriate tissue studies and clinical follow up data We believe the results justify our enthusiasm for this technique which represents an advance in our diagnostic methods and has the potential to improve our ability to detect early endometrial malignancies and their atypical precursors

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From the Department of Pathology and Cytology Southern California Permanente Medical Group and Kaiser Foundation Hospitals Los Angeles and North Hollywood California

Difficulties in the Cytologic Diagnosis of the Cold Thyroid-Nodule-Related Lymphocytic Thyroiditis

Sudha R Kini M D
J Martin Miller M D
Joel I Hamburger M D

Lymphocytic (Hashimoto s) thyroiditis may be manifested by discrete palpable thyroid nodules Diagnosis by aspiration cytology presents three types of problems (1) distinguishing the nuclear atypia of follicular cells in thyroiditis from that of follicular carcinoma (2) distinguishing the lymphoid cells of thyroiditis from those of lymphoma and (3) avoiding sampling errors due to the coexistence of lymphocytic thyroiditis and neoplasm

In a study of smears from 1 500 patients with cold thyroid nodules 120 single or combined diagnoses of lymphocytic thyroiditis were or should have been made Six patients who were suspected cytologically of having follicular adenocarcinoma were subsequently proved to have only lymphocytic thyroiditis Of 13 patients diagnosed cytologically as having an epithelial lesion composed of atypical cells nine were proved to have only lymphocytic thyroiditis and four were confirmed histologically as having follicular adenoma coexisting with lympho cytic thyroiditis Seven cases of papillary adenocarcinoma with coexisting lymphocytic thyroiditis were correctly diagnosed cytologically and confirmed histologically Of eight patients with thyroidal or perithyroidal lymphoma four were incorrectly diagnosed cytologically as having thyroiditis

All of the above disease entities and combinations thereof are illustrated and the interpretative problems are demonstrated and discussed

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From the Department of Pathology and the Division of Endocrinology Henry Ford Hospital and Northland Thyroid Laboratory Detroit Michigan

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Pseudoepidemic of Cervical Dysplasia in a Family Planning Clinic

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Elizabeth Plott C T (ASCP) C M I A C
Robert Boughton
Douglas Birdsong

The director of a community family planning clinic in Texas notified the CDC in September 1978 of an unexpected rise in the cervical dysplasia rate in clinic patients during July and August 1978 Cervical dysplasia was diagnosed from Papanicolaou smears Our investigation revealed that the cervical dysplasia rate rose from an average of 2 2% during the first six months of 1977 to an average of 6 4% from July 1977 through July 1978 nearly a threefold increase Two samples of Papanicolaou smear slides were reviewed from this clinic one from before July 1 1977 and the other from after July 1 1977 We discovered that the percentage of Papanicolaou smears with acceptable cellular sampling of the transitional zone of the cervix increased threefold after July 1 1977 A review of clinic records indicated that a key change in clinic personnel responsible for performing Papanicolaou smears had occurred in July 1977

We concluded that (1) the dysplasia rates in patients seen prior to July 1977 were artificially low due to inadequate specimens (2) the dysplasia rates observed after July 1977 were accurate and (3) no real increase in the dysplasia rate in this clinic population had occurred We suggested that the proficiency of clinic personnel be evaluated and the sensitivity of Papanicolaou smears be maximized by regular monitoring of Papanicolaou smear slide quality This investigation illustrated the first pseudoepidemic of cervical dysplasia associated with the proficiency of clinic personnel

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From the Center for Disease Control Atlanta Georgia

The Significance of the Presence of Actinomycetes in Papanicolaou Smears of IUD Users

Anisa Kanbour M D M I A C
James Harger M D
Joan Ryan C T (ASCP)
Bernard Kilonsky M D M I A C

In 1954 Hanf and Hanf reported the presence of actinomycetes on routine examination of the vaginal flora in 4% of women with poor vaginal hygiene Recently the association of actinomycetes with the use of IUDs has been widely documented It has been recommended that when these organisms are recognized in vaginal smears the IUD be removed and/or antibiotic therapy be administered

A study to define the population of IUD users colonized by actinomycetes and the optimal management of such patients was undertaken at Magee Womens Hospital Cervicovaginal smears from 330 patients using an IUD were reviewed Actinomycetes like organisms were present in 70 cases (22%) An additional 150 patients with actinomycetes like organisms were identified from current cytologic material In 50 patients Papanicolaou smears of previous years were available for review Actinomycetes like organisms were present in previous Papanicolaou smears of 26 of 50 patients (52%) for a period ranging from one to six years Clinically none of these patients required medical intervention

These findings indicate that these are opportunistic organisms their presence in the lower genital tract does not necessitate drastic therapy

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From the Magee Womens Hospital Pittsburgh Pennsylvania

A Gingivalis-Like Entamoeba *in the Genital Tracts of IUD Users*

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Ruth Etta McNeill C T (ASCP)
John K Frost M D F I A C
Prabodh K Gupta M D F I A C
Louis S Diamond Ph D
Bronislau M Honigberg Ph D

Entamoeba histolytica vulvovaginitis and cervicitis are well known clinical complications of intestinal amebiasis in hyperendemic areas However the finding of genital amebic trophozoites in association with IUD usage has been only recently reported by two of the authors (R E M and M M R) in a resident of Baltimore Maryland The clearing of these organisms following the removal of the IUD inspired the authors to further investigation

Fast smears from 947 IUD wearers and an additional control group of 1 164 Fast smears from women in their reproductive age but with no history of wearing an IUD were examined microscopically All specimens were stained by the Papanicolaou technique In addition iron hematoxylin and Gomori Wheatley trichrome stains were used in repeat specimens of patients found to harbor amebic trophozoites

Twelve additional women harboring amebae in their genital tracts were found All of them were IUD wearers and colonies of actinomycetes were also present in their Fast smears Follow up cytology studies were available in nine out of the 13 cases and there was complete clearing of these two organisms following the removal of the IUD

A few attempts to culture the ameba have not been successful Morphology studies IFA studies and the clinical course have led the authors to believe the trophozoites were *E gingivalis* like

Discussion includes characteristic morphology of the ameba clinical data and the possible mode of transmission

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From the Cytopathology Unit U S Public Health Service Hospital and the Johns Hopkins Hospital Baltimore Maryland the Laboratory of Parasitic Diseases National Institutes of Health Bethesda Maryland and the University of Massachusetts Amherst Massachusetts

Fine Needle Aspiration Biopsy of the Breast
Diagnostic Pitfalls

Tilde S Kline M D M I A C

More than 4 000 outpatient aspiration biopsies from the breast were taken Of them 3 676 were from benign lesions as verified by histologic section in 1 000 cases and repeated visits mammography and additional aspiration biopsies in the remainder In 65 (1 8%) patients with benign disease cells in the aspirate appeared worrisome and formal biopsy was suggested In all of these cases frozen section was deemed mandatory The 65 cases included fibrocystic disease fibroadenomata lobular hyperplasia with pregnancy and papillomata Representative illustrations of these diagnostic pitfalls are shown

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From the Department of Pathology Lankenau Hospital Philadelphia Pennsylvania analytical features based on cell morphology was extracted and combined mathematically to be expressed as a single number (atypia status index) This index represented the stages of the cellular abnormality (metaplasia mild moderate or severe atypia carcinoma) quantitatively as a single number varying between 0 0 and 5 5 The atypia status index for each cell correlated highly (94% to 99%) with visual cytopathologic classification The distribution of cells according to their indices was profiled for patients at various stages of carcinogenesis and was used to classify patients relative to their degree of cell atypia These results compared favorably to the clinical evaluation

This work was supported in part by NCI Contract NO1 CP-65837

From the Cellular Analytical Laboratory Johnson Space Center and the Department of Pathology Baylor College of Medicine Houston Texas

Altered Kinetics of Human Breast Carcinoma in Axillary Lymph Node Metastases

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Zbigniew Darzynkiewicz M D
M L Claps M D
Myron R Melamed M D F I A C

A number of reports suggest that the clinical course of human breast carcinoma may be more favorable in patients with a reactive hyperplasia of regional lymph nodes or lymphocytic infiltration of the primary tumor The mechanism is unknown but is presumed to involve cellular immunity In flow cytometry studies of human breast cancer we have noted in about one third of the patients an apparent change in the cell cycle kinetics of tumors metastatic to axillary lymph nodes when compared with the primary neoplasm In these cases there was a decrease in the proportion of cells in S and G_2+M There was no difference in ploidy level This observation adds to the evidence of an interaction between lymphocytes and tumor cells and suggests that lymphocytes may be involved in the control of tumor growth

This work was supported in part by Memorial Sloan Kettering Cancer Grant 1161015 from the Brian Piccolo Research Fund Dr Olszewski is an Alfred Jurzykowski Foundation Fellow

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From the Cytology Service Department of Pathology Memorial Sloan Kettering Cancer Center New York New York

A New Cervical Cell Classification System Validated by Automated Measurement

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Claire McLatchie C T (ASCP) C M I A C
Ben White Ph D
Kenneth R Castleman Ph D

We have developed an expanded cell classification system for use in automated cytology experiments The cell classes include both the range of normal physiologic variation and the gradation of change in the natural history of cervical carcinogenesis We have used a computer-controlled light microscope and a digital image processing system to extract a variety of size shape density and texture measurements from 7 000 normal and abnormal cervical cells visually classified according to our new system We found a direct relationship between the gradient of our cell classes and the values of several of the automated cell measurements The agreement constitutes an objective validation of our empirical cell classification system It also suggests that the machine measured cell features represent important sequential cellular events in the developmental stages of cervical cancer There is also the potential that an automated screening system by providing case profiles of cell classes may contribute to the standardization and accuracy of the diagnostic process

This paper represents results of research supported in part by NCI/NASA Inter Agency agreement #70314 between the National Cancer Institute and the National Aeronautic and Space Administration

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From the School of Public Health and the Cytology Laboratory UCLA School of Medicine Los Angeles and the Jet Propulsion Laboratory Pasadena California

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Models to Demonstrate Fourier Transforms of Nuclear Size Pleomorphism and Hyperchromasia

Charles P Miles M D
Dwight L Jaggard Ph D

The optical Fourier transform of an object is proportional to the object s diffraction pattern For example the diffraction pattern of a slit is perpendicular to the slit edges and consists of a series of decreasing maxima and minima (points) The diffraction pattern of a square is similar to that of two slits at right angles to each other whereas the diffraction pattern of a circle consists of concentric rings

Fourier transforms have been used empirically in attempts to distinguish benign cells from cancer cells This approach does not reveal the desired correlation between specific features in the optical Fourier transform and specific features of the cell under investigation We have used nuclear models to examine the effect of nuclear size and pleomorphism on the Fourier transform Since benign cells more often have smooth oval nuclei whereas cancer cells have more angular nuclei with sharp corners and straight edges we have investigated the Fourier transforms of corresponding models The resulting diffraction patterns are distinctly different the patterns of benign models consist of light which is relatively constant in the azimuthal direction whereas the patterns of cancer models consist of large radial spikes of light Nuclear size can be determined from the fact that nuclear dimensions are inversely proportional to corresponding transform dimensions Our initial studies show definable differences between coarsely clumped chromatin and finely divided chromatin

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From the Department of Pathology University of Utah Medical Center and the Department of Electrical Engineering University of Utah Salt Lake City

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Surface Configuration of Human Tumor Cells Obtained by Fine Needle Aspiration Biopsy

A Comparative Light Microscopic and Scanning Electron Microscopic Study

Wenancjusz Domagala M D M I A C
Leopold G Koss M D F I A C

The surfaces of viable cells of 30 primary and metastatic human malignant tumors of various origins (carcinomas of the breast and the lung malignant melanomas malignant lymphomas) were studied by scanning electron microscopy The cells were obtained by fine needle aspiration biopsy and were identified by light microscopy prior to the scanning electron microscopic examination There was no evidence of a specific surface configuration for any given tumor type On the contrary it has been shown that surface configuration of tumor cells of the same primary origin differs considerably from case to case The possible significance of these findings is discussed

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Letters to the Editors

Human Papilloma Virus Infection of the Uterine Cervix

To the Editors

The characteristic cytologic patterns in the presence of condylomatous lesions of cervix and vagina have been described [6,7,9] The cellular findings consist principally of two important features koilocytosis and dyskeratosis The presence of virus particles in koilocytotic cells reprocessed from a smear has been reported [3] We would like here to confirm those findings and add new information since the same particles were also observed in dyskeratotic cells of flat condylomata of the uterine cervix

Seven smears of flat condylomatous lesions obtained by colposcopy and diagnosed by cytology were reprocessed for electron microscopy according to the method described by Coleman et al [1] Virus particles were observed in three of seven cases All three smears contained koilocytotic cells and one also showed dyskeratotic cells

The koilocytotic cell (Figure 1) is easily recognized at the ultrastructural level since it presents a large clear space free of cytoplasmic organelles close to the nucleus Tonofilaments are abundant and coarsely clumped in the outer zone of cytoplasm giving a supporting framework to the cell

In contrast the cytoplasm of dyskeratotic cells (Figure 2) contains many thick bundles of tonofibrils in which no individual tonofilaments are distinguishable Cytoplasmic organelles are inconspicuous they probably disappear as keratinization progresses

Marginated clumped chromatin seen in both dyskeratotic and koilocytotic cells (Figures 1 and 2) surrounds virus particles freely scattered everywhere in the nucleus In one nucleus virus particles were assembled in a crystalline arrangement (Figure 3) Most frequently only a few virus particles were found within the nuclei but sometimes they occupied the entire nucleus Only a few nuclei of koilocytotic and dyskeratotic cells contained virus particles Where the nuclear envelope had ruptured virus particles could be seen around the broken membrane and everywhere in the cytoplasm as well as close to the cytoplasmic envelope Virus particles were also observed in the upper layer of the epithelium of thin sections of corresponding cervical biopsy specimens as described previously [2,5]

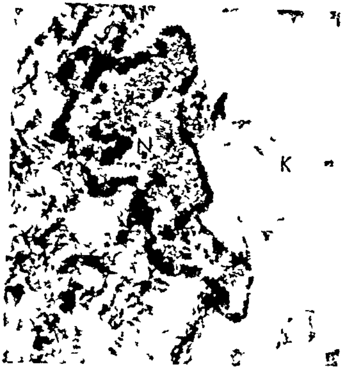

Figure 1
Electron micrograph of a koilocytotic cell with a large perinuclear space (K) devoid of cytoplasmic granules (× 7 500)

The particles themselves (Figure 4) have a regular spherical outline composed of a dense core with surrounding less dense material Their average diameter ranges from 45 to 50 nm which is the usual size in thin section of human papilloma viruses (HPV) a subgroup of the *Papovaviridae*

The finding of human papilloma viruses in koilocytotic and dyskeratotic cells strengthens the cytologic diagnosis of flat condyloma The presence of these cells in smears can be considered a marker for this venereally transmitted disease

The presence of papilloma viruses in cervical condylomatous lesions is of great significance The possi

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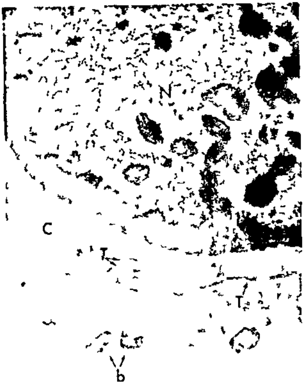

Figure 2
Ultrathin section of a dyskeratotic cell with bundles of tonofibrils (t) in the cytoplasm (c) and a nucleus (n) entirely filled with virus particles Bacteria (b) may be seen close to the cell (× 11 000)

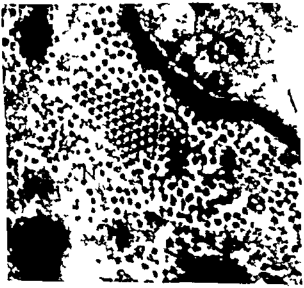

Figure 3
Part of the nucleus (n) of the cell shown in Figure 1 with margination of the chromatin and a crystalline arrangement of virus particles (× 3 000)

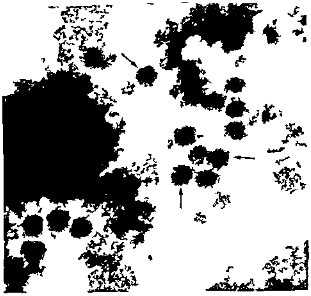

Figure 4
Electron micrograph of intranuclear virus particles showing a dense core with surrounding less dense material (× 125 000)

bility that HPV may play a role in the development of carcinoma of the cervix is suggested by the fact that condylomata have been found to transform into squamous carcinoma on the vulva and penis Epidemiologic evidence shows that carcinoma of the cervix behaves like a venereally transmitted disease[6] and condylomata of the cervix have proven to be much more frequent than formerly suggested but are often misdiagnosed morphologically as dysplasia[7] For all these reasons carcinoma of the uterine cervix represents a good model for the study of viral carcinogenesis and HPV is at this time one of the foremost candidates as a potential human oncogen[8] at the level of the uterine cervix

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References

1 Coleman DV Russell WJI Hodgson J Pe T Mowbray JF Human papilloma virus in Papanicolaou smears of urinary sediment detected by transmission electron microscopy J Clin Pathol 30 1015 10 0 1977

Della Torre G Pilotti S de Palo G Rilke F Viral particles in cervical condylomatous lesions Tumori 64 549 553 1978

3 Hills E Laverty CR Electron microscopic detection of papilloma virus particles in selected koilocytotic cells in a routine smear Acta Cytol 23 53 56 1979

4 Kessler II Human cervical cancer as a venereal disease Cancer Res 36 783 791 1976

5 Laverty CR Russell P Hills E Booth N The significance of noncondylomatous wart virus infection of the cervical transformation zone A review with discussion of two illustrative cases Acta Cytol 22 195 201 1978

6 Meisels A Fortin R Condylomatous lesions of the cervix and vagina I Cytologic patterns Acta Cytol 20 505 509 1976

7 Meisels A Fortin R Roy M Condylomatous lesions of the cervix II Cytologic colposcopic and histopathologic study Acta Cytol 21 379 390 1977

8 Meisels A Morin C The condyloma virus as a potential human oncogen Contemp Obstet Gynecol (in press)

9 Purola E Savia E Cytology of gynecologic condyloma acuminatum Acta Cytol 21 26 31 1977

The Presence of Mast Cells in Eosinophilic Pleural Effusions

To the Editors

To the best of our knowledge the presence of mast cells (tissue basophils) in eosinophilic pleural effusions (EPEs) has not been reported previously We observed it recently however in a case of EPE associated with squamous cell carcinoma of the lung

A 42 year-old male was admitted to the Himeji Red Cross Hospital on June 22 1978 with cough and chest pain There was no history of allergy or respiratory complaints in him or his family On admission to the hospital his temperature was 37 5 C and on physical examination there was evidence of an effusion in the lower third of the right chest It was confirmed by a roentgenogram No pulmonary parenchymal lesions were visible There was no lymphadenopathy Tuberculin test was strongly positive No ova or parasites were found on stool examination The white blood cell count on the day of admission was 7 500 per cm with 1% eosinophils

A diagnostic thoracentesis was done and 50 ml of turbid brown fluid was withdrawn The fluid had a specific gravity of 1 033 a protein level of 4 80 gm/100 ml and a nucleated cell count of 17×10^4 per cm of which 70% was eosinophils 8% mast cells and 15% neutrophils (Figures 1 and 2) The mast cells were demonstrated by acidified toluidine blue[6] and Hansel s stain[6,7] Pleural effusion smears were negative for pathogens and acid fast bacilli and subsequent cultures were also reported to be negative There were no malignant cells in the pleural fluid Total protein, albumin and individual fraction of globulins were all normal by serum electrophoresis

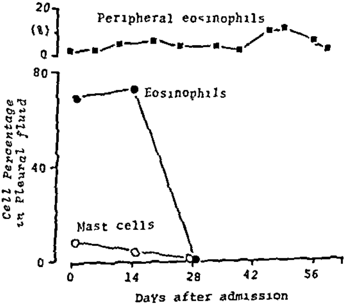

Figure 1
Comparison of the percentage of eosinophils and mast cells in the pleural fluid and in the peripheral blood The third fluid sample contained neither eosinophils nor mast cells

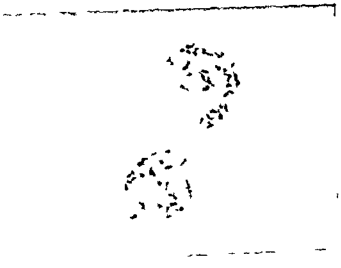

Figure 2
Mast cells with large cytoplasmic granules in pleural fluid (toluidine blue stain × 400)

Immunoglobulin of the patient was quantitated The results (and normal values) of immunoglobulin determinations were IgG 1 235 mg/100 ml (normal 1 200 mg/100 ml) IgM 120 mg/100 ml (normal 120 mg/100 ml) IgA 252 mg/100 ml (normal 210 mg/100 ml) and IgE 650 IU/100 ml (normal under 1 000 IU/100 ml) Quantitative analysis of immunoglobulin in the pleural effusion was not performed

Three weeks after admission a second thoracentesis was performed The fluid had a specific gravity of 1 034 a protein level of 4 20 gm/100 ml and a nucleated cell count of 11×10 per cm of which 73% was eosinophils 4% mast cells and 23% lymphoid cells (Figure 1) On the 28th hospital day an enlargement of the right hilus was noted Radiation therapy (200 rads/day) was started without pathologic diagnosis

On the 30th hospital day thoracentesis was performed again This fluid contained small amounts of lymphoid cells but eosinophils and mast cells were absent (Figure 1) One week after the third thoracentesis malignant cells of the squamous type were observed on routine sputum cytology The patient was placed on chemotherapy with antitumor agents Three months later the size of the tumor was reduced and the dosage of antitumor agents was also reduced

EPE is a well known entity[2 5 10] which is known to occur in association with a wide variety of disorders including infection trauma hypersensitivity and malignancy it may also occur without known etiology (idiopathic EPE) In 1964 Campbell[3] reported 101 cases of this disorder with only six cases associated with malignancy In a more recent report Veress et al observed six patients with malignancy among 30 patients with EPE

The pathogenesis of EPE is not well understood There is no correlation between blood and fluid levels of eosinophils as noted by prior investigators Mast cells (tissue basophilic granulocytes) are known to have receptors for immunoglobulin E (IgE) and cytoplasmic granules which contain histamine and heparin The coexistence of eosinophils with mast cells in the pleural fluid was an interesting laboratory finding It may be that eosinophilic chemotactic factors are related to the mast cell population in pleural fluids in EPE

Recently Beekman et al[1] and Kumar et al[7] reported on posttraumatic EPE with elevated IgE concentrations in the pleural fluid and serum Unfortunately the analysis of cellular elements in these pleural fluids was not reported In the present case no elevation of IgE was detected in the serum

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References

1 Beekman JF Bosniak S Canter HG Eosinophilia and elevated IgE concentration in a serous pleural effusion following trauma Am Rev Respir Dis 110 484-489 1974

2 B wer G Eosinophilic pleural effusion Am Rev Respir Dis 95 746 751 1967

3 Campbell GD Webb WR Eosinophilic pleural effusion Am Rev Respir Dis 90 194 01 1964

4 Cont no CA Vance JW Eosinophilic pleural effusion NY State J Med 66 044 048 1966

5 Curran WS Will ams SW Eosinophilic pleural effusion Arch Intern Med 111 809-813 1963

6 Hansel FK Clinical Allergy St Louis CV Mosby 1953 pp 408-419

7 Kumar UN Varkey B Mathai G Posttraumatic pleural-fluid and blood eosinophilia JAMA 234 625-6 6 1975

8 Parwaresch MR The Human Blood Basophil New York Springer Verlag 1976 pp 101 165

9 Sheldon JM Lovell PG Mathews KP A Manual of Clinical Allergy Philadelphia WB Saunders 1967 pp 72 77

10 Veress JF Koss LG Schreiber K Eosinophilic pleural effusions Acta Cytol 23 40-44 1979

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Editorial

The Attack on the Annual Pap Smear'

Leopold G Koss M D F I A C

Annual cytologic screening for cancer of the uterine cervix has become deeply ingrained in American society over the past quarter century The concept of the annual Pap smear presumably originated with the American Cancer Society which has been actively involved in the advocacy of this test National Center for Health Statistics data cited by Marx indicate that approximately 56 million Pap smears were performed in the United States in 1974 at an approximate cost of $200 million to $250 million Now this procedure is under attack

In 1978 Foltz and Kelsey' published a major paper critically reviewing the origin implementation and value of the annual Pap test This paper warrants careful reading because it addresses some of the important public health issues connected with the annual administration of a popular cancer prevention procedure The issues raised concern the cost of the procedure to society and its effectiveness or the cost benefit ratio Is the annual expenditure of a quarter of a billion dollars worthwhile? Does the Pap smear save lives? Who should be screened and how? What is known about the reliability of the procedure as a cancer detection device?

The Foltz and Kelsey message did not go unnoticed A few months ago *Newsweek* the popular weekly publication with a wide readership spoke of Flap with Pap More important however the

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theme was recently reviewed by the prestigious *Science* under the title The Annual Pap Smear An Idea Whose Time Has Gone? [9] The writer Jean L Marx, concluded her remarks with a gingerly endorsement of the test per se but shared some of the misgivings expressed by Foltz and Kelsey She complained of the lack of guidance on the part of the National Cancer Institute in this matter and cited extensively from the Walton report [13] which proposed a number of policies for screening women for cervical cancer in Canada

It is obvious that the issues concerning the cervical smear are of particular interest to the readers of *Acta Cytologica* and to members of the many affiliated national societies of cytology It is equally obvious that the matter of the effectiveness of the Pap smear and its cost benefit ratio must be carefully considered I have repeatedly discussed some of these problems in public forums without much response Because a large volume of cervical smears is now in the hands of profit-oriented laboratory organizations there is much vested interest in the continuation of the status quo

Unfortunately the answers to some of the questions raised by the lay writers (Foltz Kelsey and Marx are not physicians) are extremely complex Is the Pap smear cost effective? The answer clearly depends on the definition of cost effectiveness and the economic factors governing this definition Even the Walton report perhaps the most scholarly discussion of cervical cancer screening programs to date avoids this issue and states that the economic aspects of screening for cancer of the cervix would be out of place in this report The overall huge cost of the annual Pap smear to American society is dispersed because the procedure is not covered by a central insurance system There is no information available on the actual cost of some of the most successful programs such as those conducted in British Columbia [3] Jefferson County Kentucky [1] and Iceland [7] all of which have demonstrated that cytologic screening reduces the rate of invasive cancer of the cervix The gift of life to a woman is obviously priceless The Foltz and Kelsey question How much does it really cost and can the Society afford it? cannot be answered authoritatively without supporting fiscal data and the consideration of the ethical and moral factors which enter into the equation

Can the cost be reduced by spacing the screening procedure at least for women not at risk as suggested by the Walton report? It seems reasonable to assume that the socially stable woman with limited sexual exposure who had three prior negative smears is at an extremely low risk of developing cervical cancer and could be safely screened every second or third year It seems equally reasonable to assume that a socially unstable promiscuous woman should continue to have annual screenings When I made this suggestion at a recent talk in Ohio a member of the audience objected stating that in his community there were no low risk women Obviously the annual Pap smear is the one procedure in cancer prevention that has gained a high level of social and professional acceptance The annual procedure offers some additional intangible benefits such as the annual attention to health matters in general Are the risks of obliterating this accepted discipline by a less structured approach worthy of the fiscal savings? At this time there is no information on the results of the Walton recommendations and none can be expected for several years The initial results of the Swedish experience limited for fiscal reasons to smears obtained every four years cast some doubt on the wisdom of this approach [11]

Is the consumer i e the screened woman receiving good value for her money? In other words is she buying a product (in this case a cytologic diagnosis) that is of top quality and reliability? Does the smear as submitted to the laboratory faithfully reflect the status of the epithelium? There are no fully reliable objective criteria to identify an adequate smear The presence or absence of endocervical cells is in my judgment a poor criterion of adequacy because it depends not only on the method used to obtain the smear but also on the age of the patient and other clinical circumstances The use of ancillary procedures such as the endocervical aspiration has not gained broad acceptance among clinicians and its overall contribution to the diagnosis of precancerous lesions of the cervix must be considered modest

Regardless of these considerations how reliable is the Pap smear? A consensus has been developing that a single smear carries with it an unspecified but significant margin of error [2] It has been shown that two simultaneous smears are better than one [12] Are three smears better than two? What is the cost effectiveness of multiple smears? What is gained and what is lost by this approach? A truly controlled scientific study of the effectiveness of the cervical smear using optimal cytologic and colposcopic techniques and a long term follow up of a large population of women has never been done It would be an extremely costly study and perhaps could no longer be conducted in the United States where a previously unscreened population of women would be difficult to identify Perhaps some form of partnership could be developed with another country where in exchange for the benefits of screening a long term study of this type could be initiated

Assuming the optimal quality of the smear how well is it handled by the laboratory? Anecdotal knowledge suggests that significant differences in performance and reliability exist among laboratories What about factors such as human fatigue? What is really known about the cytotechnologist s ability to perceive minor cell abnormalities after one two six or eight hours of screening? What tests do we apply to determine whether the persons to whom we entrust the screening and diagnotic tasks have the talent ability and stamina to identify cell changes? There is obviously much that still awaits some fundamental inquiries

A further matter that is raised by Foltz and Kelsey is the nature of the intraepithelial changes detected by smears and the natural history of the disease There is little doubt that a large percentage of precancerous lesions detected by cytology will not progress to invasive cancer during the lifetime of the bearer The question of morphologic identification of the lesions with good prognosis versus the lesions with bad prognosis still eludes us Personal experience suggests that morphology alone does not provide the answers Are there any other helpful criteria? The evidence available at this time based on sophisticated studies of DNA distribution gives us confusing answers One study based on evaluation of the DNA content of cells in smears strongly suggested the inability of the human observer to identify the future behavior of the precancerous lesions [10] A similar study based on tissue samples reached the opposite conclusion [5]

Population studies have repeatedly shown that the rate of invasive cancer of the uterine cervix drops in the competently screened populations [1,3] Yet in some countries the effect of screening apparently cannot be translated into a clear social and health benefit In Sweden evidence has been presented that invasive cancer of the cervix may occur in previously screened populations as a consequence of inadequate laboratory and clinical handling of patients [11] Available data suggest that this experience is not confined to Sweden and has also been observed in the United States[8]—obviously a matter of great concern

These and related matters not specifically mentioned in this brief overview are obviously worthy of further study Regardless of some opinions to the contrary [6] none of the questions raised have seriously undermined the basic premise of cervical cytology namely that well run programs of cytology screening for precursor lesions are effective in reducing mortality from invasive carcinoma of the uterine cervix However in the climate of consumer inquiry in which we live it may prove worthwhile to review critically the accepted customs and to try to provide some answers to the questions raised

References

1 Christopherson WM Scott MA Trends in mortality from uterine cancer in relation to mass screening Acta Cytol 21 5 9 1977

2 Coppleson LW Brown B Estimates of the screening error rate from the observed detection rate in repeated cervical cytology Am J Obstet Gyn col 119 953 958 1974

3 Fidler HK Boyes DA Worth AJ Cervical cancer detection in British Columbia J Obstet Gynaecol Br Commonw 75 392 404 1968

4 Foltz AM Kelsey JL The annual Pap test A dubious policy success Health and Society 56 476-462 1978

5 Fu Y S Robboy SJ Prat J Nuclear DNA study of vaginal and cervical squamous cell abnormalities in DES exposed pregnancy Obstet Gynecol 52 129 137 1978

6 Green GH Cervical cancer and cytology screening in New Zealand Br J Obstet Gynaecol 85 881 886 1978

7 Johannesson G Geirsson G Day N The effect of mass screening in Iceland 1965 1974 on the incidence and mortality of cervical carcinoma Int J Cancer 21 418-425 1978

8 Lawrence CE Use effectiveness of the Pap test Presented at the Third Annual Meeting of the American Society of Preventive Oncology March 8 1979

9 Marx JL The annual Pap smear An idea whose time has gone? Science 205 177 178 1979

10 Nas ell K Auer G Nas ell M Zetterburg A Retrospective DNA analyses in cervical dysplasia as related to neoplastic progress or regression Analyt Quant Cytol 1 103 106 1979

11 Rylander E Cervical cancer in women belonging to a cytologically screened population Acta Obstet Gynecol Scand 55 361 366 1976

12 Sedlis A Walters AT Bal n H Hontz A Lo Sciuto L Evaluation of two simultaneously obtained cervical cytological smears A comparison study Acta Cytol 18 291 296 1974

13 Walton report Cervical cancer screening programs Can Med Assoc J 114 1003 10 6 1976

Spindle and Giant Cell Carcinoma of the Thyroid

Cytologic Diagnosis by Fine Needle Aspiration

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Eight patients with spindle and giant cell carcinoma of the thyroid were seen between 1973 and 1977 at the Medical College of Virginia representing 12% of all neoplasms of the thyroid in that period The mean age at detection was 69 2 years The mean survival was 5 3 months Fine needle aspiration was performed in six of these eight patients In all six patients the diagnosis could be made cytologically The cytologic characteristics of this lesion are presented and the differential diagnosis is discussed

Spindle and giant cell carcinoma of the thyroid is one of the most aggressive neoplasms known to occur in humans with the median survival after diagnosis usually less than six months Between 5% and 15% of all thyroid malignancies are reported to be of the spindle and giant cell variety [1,12,14,17,19] In contrast to the extremely poor prognosis of this tumor type the majority of malignant lesions of the thyroid i e papillary and follicular carcinomas have a slow and protracted course over many years and are only rarely fatal

Fine needle aspiration was introduced as a diagnostic procedure at the Medical College of Virginia in 1972 and has since gained increasing acceptance among clinicians and pathologists [5] Recently this technique has seen increased application in the United States after having been used extensively for many years in Scandinavia and some other European countries [2,4,9,13,20] Spindle and giant cell carcinoma of the thyroid represents a particularly appropriate lesion to demonstrate the advantages of this technique because this tumor may present as a medical emergency requiring rapid diagnosis The cytologic features are highly diagnostic and surgical exploration is not indicated in the majority of patients

Materials and Methods

The files of the Division of Surgical Pathology at the Medical College of Virginia were searched for cases of malignant lesions of the thyroid diagnosed in the five year period between January 1 1973 and December 31 1977 All cases of anaplastic carcinoma of the thyroid were retrieved Medical records as well as histologic and cytologic material were reviewed Four consultation cases sent in from other hospitals were excluded Survival data were provided by the Tumor Registry of the Medical College of Virginia In the time period selected 69 malignant lesions of the thyroid had been seen (Table I) Papillary carcinoma represented 58% of all malignant lesions and was the most common tumor encountered Anaplastic carcinoma was found in eight patients (11 6%) all of the cases were of the spindle and giant cell variety Small cell anaplastic carcinoma was not encountered Seven of our patients were female only one was male The average age at the time of diagnosis was 69 2 years the youngest patient was 55 years All patients presented with a rapidly enlarging mass in the neck area Four patients had a history of previous thyroid disease three of them had a history of goiter and one patient had a cold nodule One patient pre

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Table I *Histologic Distribution of Malignant Lesions of the Thyroid at the Medical College of Virginia 1973 1977 (WHO Classification)*

	Number of cases	Percent
Follicular carcinoma	16	23 2
Papillary carcinoma	40	58 0
Squamous cell carcinoma	—	—
Anaplastic carcinoma		
Spindle and giant cell type	8	11 6
Small cell type	—	—
Medullary carcinoma	5	7 2
Nonepithelial tumors	—	—
Total	69	100 0

sented in the emergency room with acute respiratory distress

Results

In six of the eight patients the diagnosis was established by aspiration biopsy The findings on these six patients are summarized in Table II In two patients histologic material became subsequently available confirming the cytologic diagnosis (Figure 1)

All cytologic slides were reviewed The results are summarized in Table III The most consistent and helpful features were the presence of necrotic debris in the background and the rich cellularity of the specimen with a mixed picture of small tissue fragments and singly occurring cells At higher power the cells showed obvious malignant features most of them were poorly differentiated Only a minority of the

Table II *Summary of Clinical Data on Six Patients with Spindle and Giant Cell Carcinoma of the Thyroid*

Patient (age sex race)	Chief complaint and clinical presentation	Cytologic diagnosis	Tissue diagnosis	Survival (months)	Comment
Case 1 61/F/W	Rapidly enlarging thyroid mass with extension into parotid area	Carcinoma—consistent with giant cell carcinoma of thyroid	Open biopsy 4 weeks previously in outside hospital chronic thyroiditis (Hashimoto)	1	No treatment
Case 2 75 F B	Large goiter for several years recently increasing presented with upper airways obstruction and severe respiratory distress	Carcinoma—papillary and undifferentiated of thyroid	None	0	Died 5 days after admission
Case 3 75 F B	Neck had grown very rapidly severe shortness of breath rapid atrial fibrillation large hard mass in left lobe and isthmus	Spindle and giant cell carcinoma of thyroid	None	2	On initial presentation 5 cm firm mass in lumbar area needle aspiration showed follicular carcinoma
Case 4 75 F W	Nontoxic colloid goiter 11 years previously now rapidly growing mass of right side of neck for 3 months	Spindle and giant cell carcinoma of thyroid	None	2	Initial decrease of mass after 6 000 rads
Case 5 55 M W	Cold nodule of thyroid 8 years previously week history of weakness anorexia diarrhea status after recent myocardial infarct and cerebrovascular accident	Spindle and giant cell carcinoma of thyroid	Autopsy 6 × 3-cm mass right lobe of thyroid necrotic histologically spindle and giant cell carcinoma no metastases cause of death *Klebsiella* sepsis	1	None
Case 6 69 F W	Swelling in neck for several weeks hoarseness right-sided thyroid mass 7 × 5 × 3 cm	Malignant cells consistent with spindle and giant cell carcinoma	Partial thyroidectomy 4-cm necrotic mass in right lobe histologically spindle and giant cell carcinoma	34	5 000 rads postoperatively 2 courses of adriamycin ICRF #159 cyclophosphamide and 5-fluorouracil

Spindle and Giant Cell Carcinoma of the Thyroid

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Eight patients with spindle and giant cell carcinoma of the thyroid were seen between 1973 and 1977 at the Medical College of Virginia representing 12% of all neoplasms of the thyroid in that period The mean age at detection was 69 2 years The mean survival was 5 3 months Fine needle aspiration was performed in six of these eight patients In all six patients the diagnosis could be made cytologically The cytologic characteristics of this lesion are presented and the differential diagnosis is discussed

Spindle and giant cell carcinoma of the thyroid is one of the most aggressive neoplasms known to occur in humans with the median survival after diagnosis usually less than six months Between 5% and 15% of all thyroid malignancies are reported to be of the spindle and giant cell variety [1,12,14,17,19] In contrast to the extremely poor prognosis of this tumor type the majority of malignant lesions of the thyroid i e papillary and follicular carcinomas have a slow and protracted course over many years and are only rarely fatal

Fine needle aspiration was introduced as a diagnostic procedure at the Medical College of Virginia in 1972 and has since gained increasing acceptance among clinicians and pathologists [5] Recently this technique has seen increased application in the United States after having been used extensively for many years in Scandinavia and some other European countries [2,4,9,13,20] Spindle and giant cell carcinoma of the thyroid represents a particularly appropriate lesion to demonstrate the advantages of this technique because this tumor may present as a medical emergency requiring rapid diagnosis The cytologic features are highly diagnostic and surgical exploration is not indicated in the majority of patients

Materials and Methods

The files of the Division of Surgical Pathology at the Medical College of Virginia were searched for cases of malignant lesions of the thyroid diagnosed in the five year period between January 1 1973 and December 31 1977 All cases of anaplastic carcinoma of the thyroid were retrieved Medical records as well as histologic and cytologic material were reviewed Four consultation cases sent in from other hospitals were excluded Survival data were provided by the Tumor Registry of the Medical College of Virginia In the time period selected 69 malignant lesions of the thyroid had been seen (Table I) Papillary carcinoma represented 58% of all malignant lesions and was the most common tumor encountered Anaplastic carcinoma was found in eight patients (11 6%) all of the cases were of the spindle and giant cell variety Small cell anaplastic carcinoma was not encountered Seven of our patients were female only one was male The average age at the time of diagnosis was 69 2 years the youngest patient was 55 years All patients presented with a rapidly enlarging mass in the neck area Four patients had a history of previous thyroid disease three of them had a history of goiter and one patient had a cold nodule One patient pre

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Table I *Histologic Distribution of Malignant Lesions of the Thyroid at the Medical College of Virginia 1973 1977 (WHO Classification)*

	Number of cases	Percent
Follicular carcinoma	16	23 2
Papillary carcinoma	40	58 0
Squamous cell carcinoma	—	—
Anaplastic carcinoma		
Spindle and giant cell type	8	11 6
Small cell type	—	—
Medullary carcinoma	5	7 2
Nonepithelial tumors	—	—
Total	69	100 0

sented in the emergency room with acute respiratory distress

Results

In six of the eight patients the diagnosis was established by aspiration biopsy The findings on these six patients are summarized in Table II In two patients histologic material became subsequently available confirming the cytologic diagnosis (Figure 1)

All cytologic slides were reviewed The results are summarized in Table III The most consistent and helpful features were the presence of necrotic debris in the background and the rich cellularity of the specimen with a mixed picture of small tissue fragments and singly occurring cells At higher power the cells showed obvious malignant features most of them were poorly differentiated Only a minority of the

Table II *Summary of Clinical Data on Six Patients with Spindle and Giant Cell Carcinoma of the Thyroid*

Patient (age sex race)	Chief complaint and clinical presentation	Cytologic diagnosis	Tissue diagnosis	Survival (months)	Comment
Case 1 61 F/W	Rapidly enlarging thyroid mass with extension into parotid area	Carcinoma—consistent with giant cell carcinoma of thyroid	Open biopsy 4 weeks previously in outside hospital chronic thyroiditis (Hashimoto)	1	No treatment
Case 75/F B	Large goiter for several years recently increasing presented with upper airways obstruction and severe respiratory distress	Carcinoma—papillary and undifferentiated of thyroid	None	0	Died 5 days after admission
Case 3 75 F B	Neck had grown very rapidly severe shortness of breath rapid atrial fibrillation large hard mass in left lobe and isthmus	Spindle and giant cell carcinoma of thyroid	None		On initial presentation 5 cm firm mass in lumbar area needle aspiration showed follicular carcinoma
Case 4 75 F W	Nontoxic colloid goiter 11 years previously now rapidly growing mass of right side of neck for 3 months	Spindle and giant cell carcinoma of thyroid	None	2	Initial decrease of mass after 6 000 rads
Case 5 55 M W	Cold nodule of thyroid 8 years previously week history of weakness anorexia diarrhea status after recent myocardial infarct and cerebrovascular accident	Spindle and giant cell carcinoma of thyroid	Autopsy 6×3-cm mass right lobe of thyroid necrotic histologically spindle and giant cell carcinoma no metastases cause of death *Klebsiella* sepsis	1	None
Case 6 69 F W	Swelling in neck for several weeks hoarseness right-sided thyroid mass 7×5×3 cm	Malignant cells consistent with spindle and giant cell carcinoma	Partial thyroidectomy 4-cm necrotic mass in right lobe histologically spindle and giant cell carcinoma	34	5 000 rads postoperatively [illegible] courses of adriamycin ICRF #159 cyclophosphamide and 5 fluorouracil

Table III *Cytologic Presentation of Spindle and Giant Cell Carcinoma of the Thyroid in Needle Aspirations from Six Patients*

	Case					
	1	2	3	4	5	6
General features						
Necrosis	++	++	+++	++++	++	++++
Tissue fragments	++	−	+	−	+++	−
Leukocytes	+	++++	++	++	++	++
Erythrocytes	++++	+	(+)	+	++	+
Cytologic features						
Cellularity	++	+++	+++	++	++++	+
Giant cells	+++	+	(+)	(+)	+	(+)
Spindle cells	(+)	(+)	+++	++	+	+
Single cells	++	+++	+++	++	++	++
Prominent nucleoli	++++	++	++	+++	++++	−

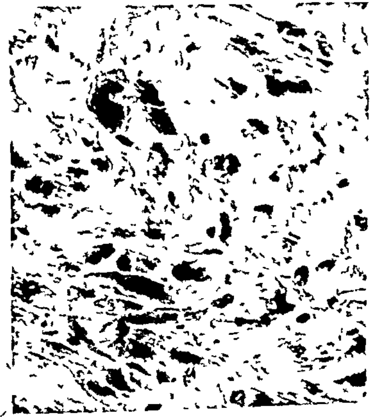

Figure 1
Tissue section of spindle and giant cell carcinoma of the thyroid (hematoxylin and eosin × 475)

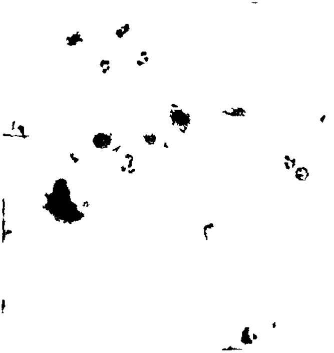

Figure 2
Aspiration cytology of spindle and giant cell carcinoma of the thyroid Several giant cells and one spindle cell are seen Note the presence of leukocytes for comparison of size note also that this figure was taken at the same magnification as Figure 1 There is necrotic debris in the background (Papanicolaou stain × 475)

cells showed the highly diagnostic spindle or giant cell features (Figures 2 and 3) Usually either the spindle or the giant cell type predominated Nucleoli were found to be prominent in all cases except case 6 Most of the material in this patient consisted of necrotic debris with a few scattered spindle cells The presence of microbiopsies or tissue fragments was a helpful feature (see Figure 3) but occurred in only half the cases

Discussion

Spindle and giant cell carcinoma of the thyroid is a lethal disease with a dismal prognosis At M D Anderson Hospital in Houston Texas spindle and giant cell carcinoma represents 7.2% of all thyroid neoplasms[1] at Memorial Hospital in New York City the ratio is 5.5%[11] and at the University of Cape Town Medical Center it is 10.2%[17] In our series of 69 cases over a five year period 12% of all malignant lesions of the thyroid were of the spindle and

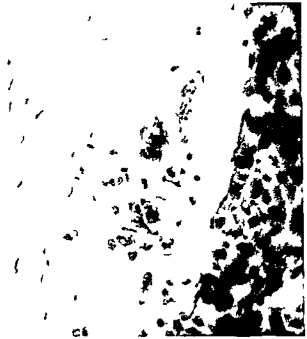

Figure 3
Aspiration cytology of spindle and giant cell carcinoma of the thyroid Singly occurring tumor cells and a tissue fragment are seen Note the presence of spindle and giant forms in this field By comparing these cells with the leukocytes present their enormous size can readily be appreciated (Papanicolaou stain × 300)

giant cell variety It is currently thought that spindle and giant cell carcinoma of the thyroid arises from a previously existing differentiated tumor through anaplastic transformation[1, 14] After thorough sectioning remaining foci of a differentiated lesion may be demonstrated in most cases[8,11] Recent reevaluations of tumors of the thyroid previously diagnosed as sarcoma led in the overwhelming majority to a diagnosis of undifferentiated carcinoma spindle cell type[8,13] The relatively high incidence of sarcoma of the thyroid reported earlier in Switzerland has considerably decreased and presently is at approximately the same level as in other parts of the world[8,15]

As to the differential diagnosis of primary thyroid tumors in needle aspirates there is general agreement that the features of spindle and giant cell carcinoma are highly specific[3,4,12-20] Likewise papillary medullary and oncocytic tumors are diagnosed reliably by needle aspiration[12-20] However the differentiation of follicular adenoma and follicular carcinoma is not considered feasible by most authors on the basis of a needle aspirate[3,12,20] This view has been challenged recently[11] Kakudo et al[10] reported a case of medullary carcinoma giant cell type as a rare entity which should be considered in the differential diagnosis of spindle and giant cell carcinoma

In the WHO classification of thyroid tumors[7] small cell anaplastic carcinoma is listed as the third type of undifferentiated carcinoma besides the giant and spindle cell varieties Although this tumor type was not seen in the material reviewed a case of histiocytic lymphoma is illustrated for differential diagnostic considerations (Figures 4 and 5) This 59 year-old female patient presented with an enlarged thyroid A needle aspirate revealed a single-cell suspension of malignant cells typically seen in lymphoma A subsequently obtained biopsy showed a small cell neoplasm The differential diagnosis of malignant lymphoma and small cell anaplastic carcinoma can be exceedingly difficult or even impossible on histologic grounds only[14] Fine needle aspiration may solve the problem since the exclusively singular occurrence of lymphoma cells as well as the occasional cluster formation of undifferentiated carcinoma cells is much more readily apparent in the aspirate

Each patient in this series presented with a history of a rapidly enlarging neck mass over the few weeks prior to admission Patient 2 presented in the emer

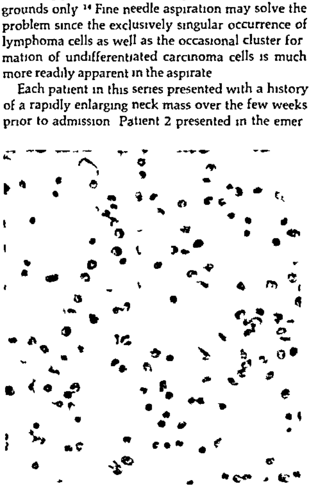

Figure 4
Aspiration cytology of malignant lymphoma histiocytic type involving the thyroid The cells show a moderate degree of pleomorphism with chromatin clumping clearing and occasional nucleoli Note the presence of erythrocytes and necrosis in the background of the smear (Papanicolaou stain × 4 5)

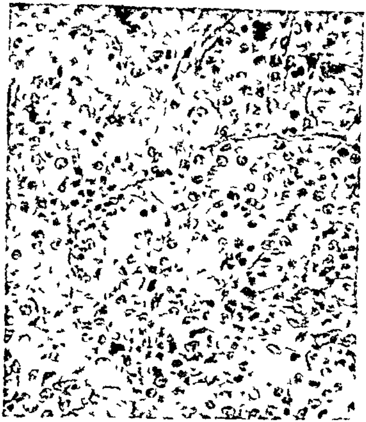

Figure 5
Tissue section of malignant lymphoma histiocytic type diffuse involving the thyroid Note that Figures 1 2 4 and 5 are taken at *the same magnification (hematoxylin and eosin X 475)*

gency room with acute respiratory distress Needle aspiration was performed in the emergency room and a diagnosis was *rendered within an hour after* admission *Likewise in four of the six patients whose* masses were aspirated *the final diagnosis* was established on the day of admission

In our experience needle aspiration has proven to be superior to needle biopsy and surgical exploration in an emergency situation when the diagnosis has to be established rapidly In addition no anesthesia is necessary for needle aspiration This represents a *particular* advantage when *dealing with a patient presenting with a large neck mass* in acute respiratory distress Finally in view of the increasing public awareness of cost containment in medical care needle aspiration is an inexpensive procedure In our institution the aspiration is performed as an outpatient or bedside procedure by the pathologist on a consultation basis There is no need for the involvement of additional personnel i e surgeon anesthesiologist or histotechnician The smears are prepared and *immediately read by the pathologist*

All patients in this series died after a mean survival of 5 3 months after diagnosis Patient 6 had a remarkably slow course and survived for 34 months

Treatment consisted of palliative resection of the primary tumor (debulking) external radiation to the thyroid with 5 000 rads and two courses of chemotherapy with 5 FU *cyclophosphamide* and adriamycin Anderson et al [2] *Rogers et al*[16] *and Sokal et al* also reported partial remission of *spindle and giant* cell carcinoma after combined chemotherapy However despite some encouraging results with vigorous combination chemotherapy *the* cure for this fatal disease is still not in sight

References

1 Aldinger KA Samaan NA Ibanez M Hill CS Jr Anaplastic carcinoma of the thyroid A review of 84 cases of spindle and *giant cell carcinoma of the thyroid* Cancer 41 2267 2275 1978

2 Andersson T Biorklund A Landberg T Åkerman M Aspegren K Ingemansson S Combined *therapy for undifferentiated* giant and spindle cell carcinoma of the thyroid Acta Otolaryngol 83 372 377 1977

3 Cornillot M Granier AM Houcke M La cyto-ponction des lésions thyroidiennes Confrontations anatomo-cytologiques I *Lesions malignes (300 observations)* Arch Anat Cytol Pathol 25 325 332 1977

4 Einhorn J Franzen S Thin needle biopsy in the diagnosis of thyroid disease Acta Radiol 58 321 326 1962

5 Frable WJ Thin needle aspiration biopsy A personal experience with 469 cases *Am J Clin Pathol 65 168 182* 1976

6 Harada T Ito K Shimaoka K *Hosoda Y Yakumaru K Fatal thyroid carcinoma Anaplastic transformation of adenocarcinoma* Cancer 39 2588 2596 1977

7 Hedinger C Sobin LH Histological Typing of Thyroid Tumours International Histological Classification of Tumours *No 11 Geneva World Health Organization* 1974

8 Heitz P Moser H *Staub JJ* Thyroid cancer A study of 573 *thyroid tumors and 161 autopsy* cases observed over a thirty year period Cancer 37 2329 2337 1976

9 Jereb B Stjernsward J Lowhagen T Anaplastic giant cell carcinoma of the thyroid A study of treatment and prognosis Cancer 35 1293 1295 1975

10 Kakudo K Miyauchi A Ogihara T Takai SI Kitamura H Kosaki G Kumahara Y Medullary carcinoma of the thyroid Giant cell type Arch Pathol Lab Med 102 445-447 1978

11 Lang W Atay Z Georgii A Die cytologische Unterscheidung follikulaerer Tumoren in der Schilddruese Virch Arch [Pathol Anat] 378 199 211 1978

12 Lieberman P Foote FW Schottenfeld D A study of the pa*thology of thyroid cancer 1930-1960* Clin Bull 2 7 12 1972

13 Lowhagen T Sprenger E *Cytologic presentation of thyroid tumors in aspiration biopsy smear* Acta Cytol 18 192 197 1974

14 Meissner WA Warren S Tumors of the *thyroid gland In Atlas of Tumor Pathology* Second series Fourth fascicle Washington DC Armed Forces Institute of Pathology 1969 pp 102 110

15 Neracher H Hedinger C Klassifizierung der Schilddrusenmalignome nach der Nomenklatur der WHO 1974 Histologische Nachkontrolle von 327 bosartigen Schilddrusentumoren Schweiz Med Wochenschr 105 1000 1006 105- 1056 1975

16 Rogers JD Lindberg RD Hill CS Jr Gehan E Spindle and giant cell carcinoma of the thyroid A different therapeutic approach Cancer 34 1328 1332 1974

17 Selzer G Kahn LB Albertyn L Primary malignant tumors of the thyroid gland A clinicopathologic study of 254 cases Cancer 40 1501 1510 1977

18 Sokal M Harmer CL Chemotherapy for anaplastic carcinoma of the thyroid Clin Oncol 4 3-10 1978

19 Woolner LB Thyroid carcinoma Pathologic classification with data on prognosis Semin Nucl Med 1 481 502 1971

20 Zajicek J Aspiration Biopsy Cytology Part I Cytology of Supradiaphragmatic Organs *In* Monographs in Clinical Cytology Edited by GL Wied Basel S Karger 1974 pp 67 89

The Canadian Society of Cytology—Societe Canadienne de Cytologie

The annual meeting of the Canadian Society of Cytology will be held with the Canadian Congress of Laboratory Medicine in St John s Newfoundland from June 21 to 25 1980

The theme of the congress this year is Hazards of Travel Various symposia are planned these include The Traveller and the Forensic Pathologist Tropical Diseases Animal Transmitted Diseases and Hemaglobinopathies There is in addition a symposium on experimental pathology and a histologic slide seminar on tropical pathology will be conducted by Dr D H Conner of the Armed Forces Institute of Pathology Washington DC

The Canadian Society of Cytology is sponsoring Dr P Alonso-deRuiz of the Hospital General SSA Mexico who will speak on cytology of tropical diseases

Twenty-one workshops are planned during the congress Both proffered papers and poster sessions are planned

For more information regarding the congress contact Dr C Hutton The Charles A Janeway Child Health Centre St John s Newfoundland Canada A1A 1R8 or Dr George Anderson Secretary Treasurer 2656 Heather Street Vancouver B C Canada V5Z 3J3

Giant Cell Carcinoma of the Lung

Comparative Studies of the Same Cancer Cells by Light Microscopy and Scanning Electron Microscopy

Akio Takenaga, M D
Minoru Matsuda, M D M I A C
Takeshi Horai, M D
Harumichi Ikegami M D
Shoji Hattori M D , F I A C

Surface ultrastructures of giant cell carcinoma cells were studied utilizing a technique developed by the authors which permits observation of the same cells by both light and scanning electron microscopy Although some general similarity was shown to exist between giant cell carcinoma and squamous cell carcinoma cells the following distinguishing features of giant cell carcinoma cells were demonstrated (1) absence of keratinization (2) full bulging cellular edges and (3) presence of deep invaginations or small hollows The features which distinguished giant cell carcinoma from adenocarcinoma cells were (1) presence of many flattened cells (2) folded cell surface (3) absence of tight clusters of cancer cells and (4) few microvilli Furthermore the characteristic granularity observed in small cell carcinoma was not observed in giant cell carcinoma cells Characteristically cells of giant cell carcinoma are extremely large and flattened engulf numerous leukocytes and have a bulging cellular edge

Giant cell carcinoma of the lung was first described by Nash and Stout [7] and its epithelial character was confirmed by Ozzello and Stout[8] in 1961 It is well known that patients with giant cell carcinoma of the lung generally show a rapid clinical course attributable to the rapid growth of the tumor and its poor response to treatment [3,4,9] Razzuk[10] stressed the necessity of bronchial brushing and transbronchial biopsy for accurate diagnosis As we reported previously [12] giant cell carcinoma often develops around the larger bronchi and instead of producing a bulky intraluminal growth it grows in the peribronchial spaces as does oat cell carcinoma Therefore the appearance of obstructive bronchial symptoms is delayed and metastases to the hilar lymph nodes are often present at the time of diagnosis This underlies the importance of the early diagnosis of the tumor by means of bronchial brushing cytology or needle aspiration cytology

Naib [6] Matsuda[5] and Broderick[2] have reported cytologic findings peculiar to giant cell carcinoma cells in sputa bronchial washing and bronchial brushing materials

Bahr[1] reported an excellent method for the study of the same cells by light microscopy (LM) and scanning electron microscopy (SEM) In a previous paper[11] we reported another technique for the same purpose

The present study was performed to compare the surface configuration of the same cells by LM and SEM in order to obtain a better understanding of the characteristic features of giant cell carcinoma

Materials and Methods

Cells were obtained by transcutaneous needle aspiration from two cases of giant cell carcinoma For the LM studies the aspirated material was first smeared on glass slides and fixed in glutaraldehyde solution (2 5 M in 0 1 M cacodylate buffer pH 7 2) for 30 minutes and stained by Papanicolaou s method The specimens were examined with the light microscope and the cells to be examined by SEM were marked

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with a circle After being kept overnight in xylol solution to remove the cover glass the marked areas were cut with a glass cutter into 5-sq mm pieces which were dried by the critical point method The specimens were then mounted on a stub using silver circuit paint and were coated with gold The procedure is summarized in Table I

As already reported[11] many malignant and nonmalignant cells were examined by the SEM technique in order to ascertain whether changes in the surface ultrastructure were produced by the Papanicolaou stain It was shown that this technique did not markedly alter the configuration of the cell surfaces

The observations were carried out with a JEOL (Tokyo Japan) JSM SI scanning electron microscope operating at 10 kv

Results

The large cells of giant cell carcinoma appeared mostly as single cells in the SEM Frequently these single cells were flattened had a rounded configuration and had a slight central depression corresponding to the cytoplasmic vacuoles seen by LM Cell surfaces appeared granular with a few microridges (Figure 1) and were sometimes slightly folded (Figure 2) The edges however were smooth full and bulging which served to distinguish them from the sharply defined cellular boundaries of squamous carcinoma cells At times hyaloplasm like substances were observed at the cellular edges (Figure 2) The tail like cytoplasmic processes which were observed on occasion closely resembled the tadpole cells of squamous cell carcinoma However there was a distinguishing feature small pits or hollows on the surface of the giant cell carcinoma cells they corresponded to the vacuoles seen by LM (Figure 3)

Round single cells with deep crater like invaginations were also observed The cell surface was not covered with numerous microvilli as is the case in adenocarcinoma but instead a few microridges were observed (Figure 4)

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Critical point drying
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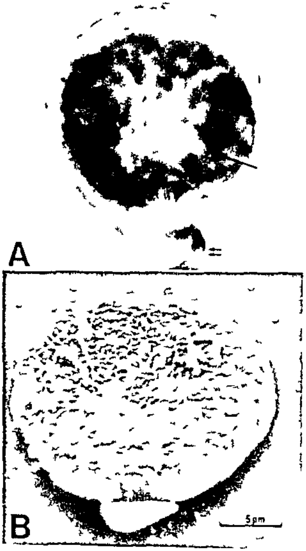

Figure 1
(A) Giant cell carcinoma cell Hazy center (arrow) corresponds to large cytoplasmic vacuole containing mucus An erythrocyte is visible (arrows) (Papanicolaou stain × 4 500) (B) SEM of the same cell Note the flattened round shape fuzzy granular surface with a few microridges and characteristically bulging edge The central concavity corresponds to the cytoplasmic vacuole (× 5 400)

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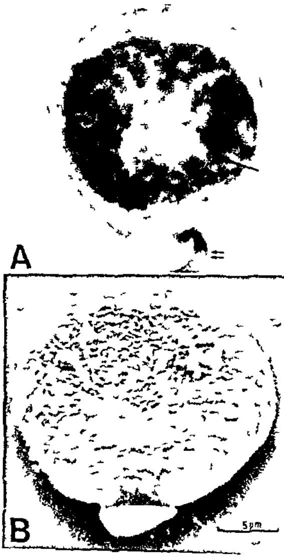

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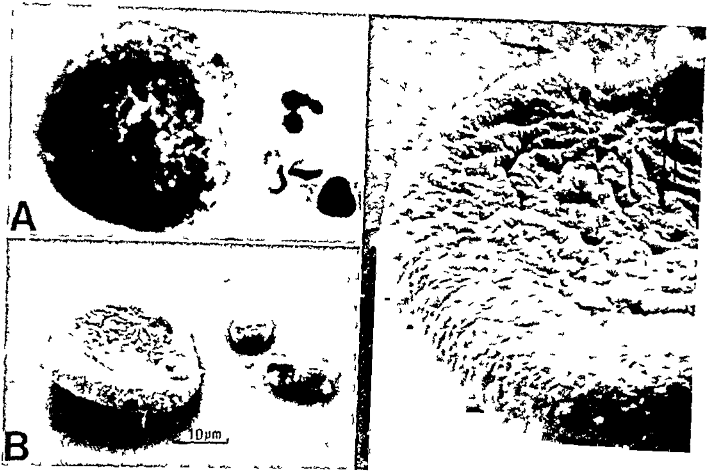


Figure 2
(A) The cell on the left dwarfs the leukocytes and erythrocytes (Papanicolaou stain × 2 500) (B) SEM of the same cell (× 2 200) (C) The cell surface is slighty folded and the edge bulges distinctively Hyaloplasm like substance can be seen (arrow) and a small pit (arrows) corresponds to the small cytoplasmic vacuole seen by LM (× 9 000)

The flattened surface of an extremely large cancer cell containing numerous leukocytes in the cytoplasm appeared uneven and slightly bumpy with the bumps corresponding to the sites of the engulfed leukocytes (Figure 5) This finding was observed in both giant cell carcinomas but so far not in other types of lung cancer cells examined by SEM

When giant cell carcinoma cells were found as a group the intercellular connections were not as tight as in clusters of adenocarcinoma cells the cells overlapped each other and their granular surfaces were covered with only a few microridges (Figures 6 and 7)

Discussion

The surface ultrastructures of giant cell carcinoma cells were studied utilizing our technique which permits observation of the same cells by both LM and SEM

Giant cell carcinoma cells generally appeared as single cells in the SEM thereby facilitating precise observation of the surface ultrastructure Ultrastructurally giant cell carcinoma cells bore some resemblance to the cells of squamous cell carcinoma (1) there were many flattened cells of large size (2) tadpole like cells were present and (3) there was occasionally pronounced surface folding At the same time however several distinguishing features peculiar to giant cell carcinoma cells were observed (1) there was an absence of curled edges and a sharply defined cellular boundary (2) the cellular edges were full bulging even in flattened cells (3) deep invaginations corresponding to the site of vacuoles seen by LM were observed and (4) small pits or hollows were present even in the surface of tadpole like cells

Cytologic examination by LM does not always permit easy differentiation between large adenocarcinoma cells which have engulfed a few leukocytes and giant cell carcinoma cells The present study showed that ultrastructural features common to both included (1) deep invagination (2) occasional small pits or hollows and (3) bulging cellular edges How

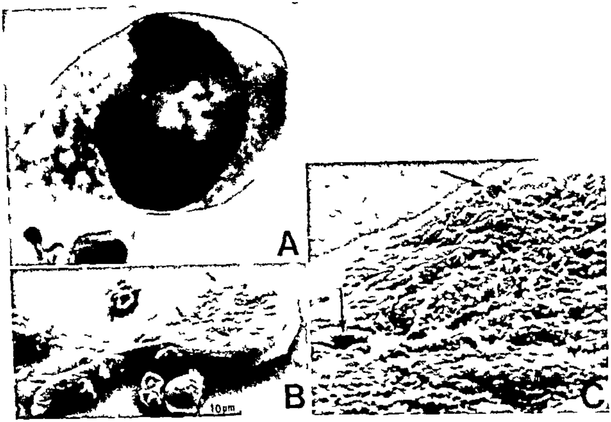


Figure 3
(A) Tadpole like giant cell carcinoma cell The hazy areas in the nucleus of this tadpole like cell represent cytoplasmic vacuoles Foamy cytoplasm is visible (Papanicolaou stain × 3 600) (B) SEM shows surface unevenness with cytoplasmic vacuoles and pits (arrows) corresponding to the most remarkable vacuoles (× 2 000) (C) Close-up of pits (× 9 400)

ever the following features of giant cell carcinoma cells were decisively different from those of adenocarcinoma cells (1) the presence of many flattened cells (2) folded cell surfaces (3) no tight clusters of cancer cells (4) few microvilli and (5) many single cells

The single most characteristic feature of giant cell carcinoma is an extremely large flattened cancer cell engulfing numerous leukocytes Unlike those of histiocytes the cellular edges of giant cell carcinoma cells were well filled bulging and smooth As we already reported small cell carcinoma cells are rounded but irregular in shape their surface is rather smooth but covered with characteristic granular deposits of various sizes This type of granular surface was not seen in cells of giant cell carcinoma It was thus demonstrated that giant cell carcinoma cells have unique ultrastructural characteristics

In our transmission electron microscopic (TEM) studies [12] we detected the presence of intracytoplasmic microcysts containing many leukocytes or cellular debris which corresponded to the cytophagocytosis suggested by LM Also the abundant mucous substances seen in the enlarged endoplasmic reticulum during our TEM study seem to correspond to the invagination and pits revealed by the present SEM study

Using light microscopy Naib [4] Matsuda [5] and Broderick [3] reported exfoliative cytology findings peculiar to giant cell carcinoma such as very large single cells multinucleated cells characterized by large nucleoli and apparent cytophagocytosis The surface configuration observed by SEM is complementary to these LM characteristics and further clarifies the cellular features of giant cell carcinoma

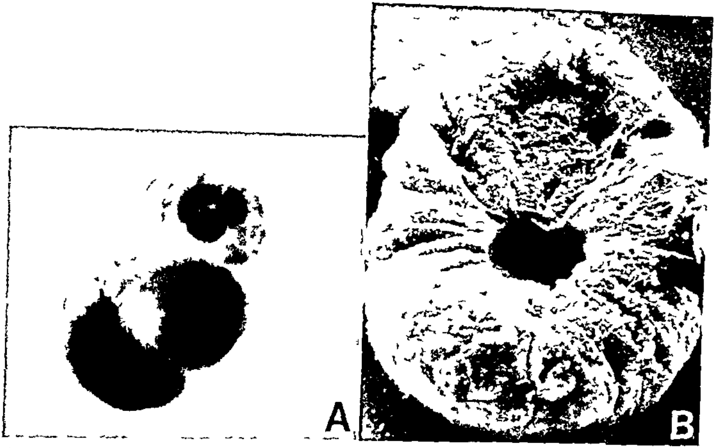
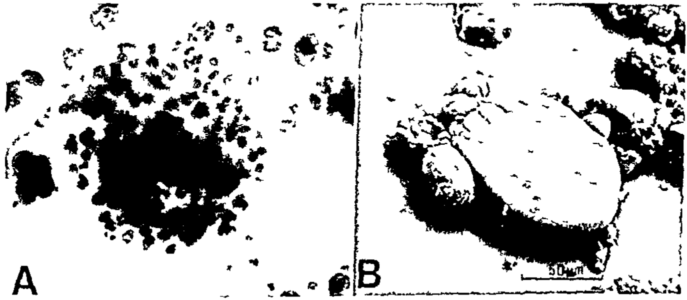


Figure 4
(A) LM shows a giant cell carcinoma cell apparently overlapped by a leukocyte (Papanicolaou stain, × 4,000). (B) SEM reveals slight folding of the cell. The central "crater-like" deep invagination corresponds to the large cytoplasmic vacuole seen by LM (× 8,500).



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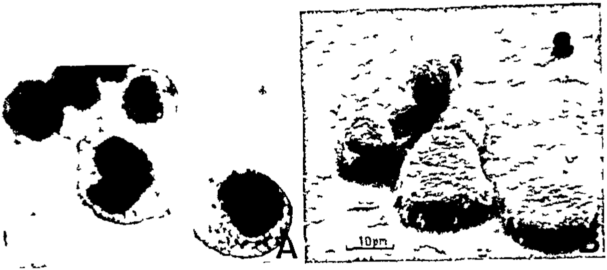

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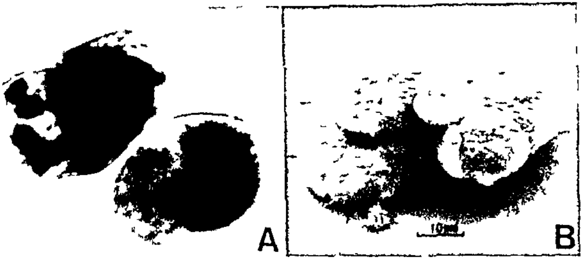

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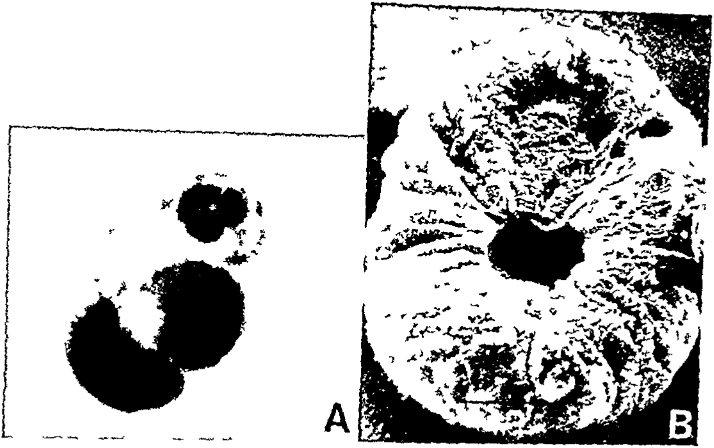

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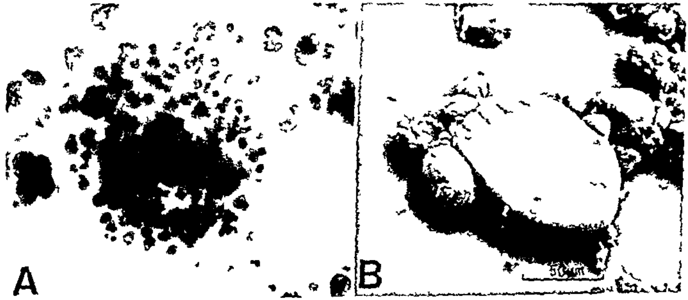

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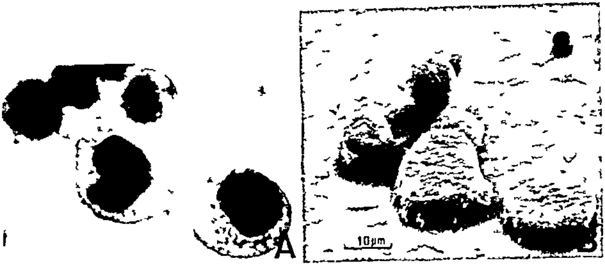

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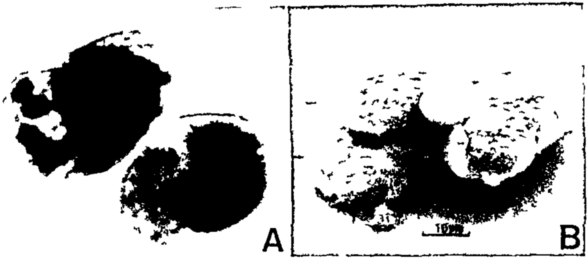

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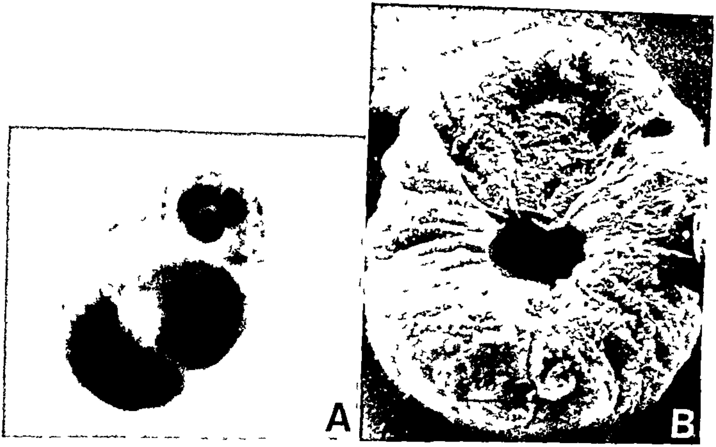

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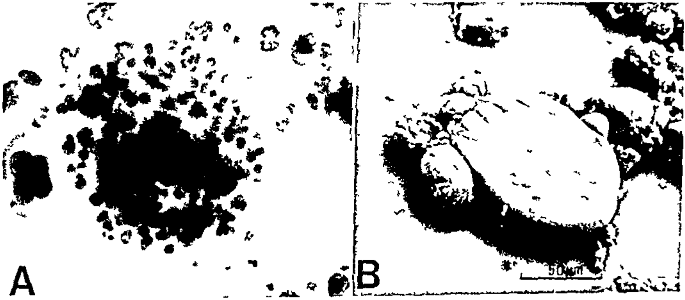

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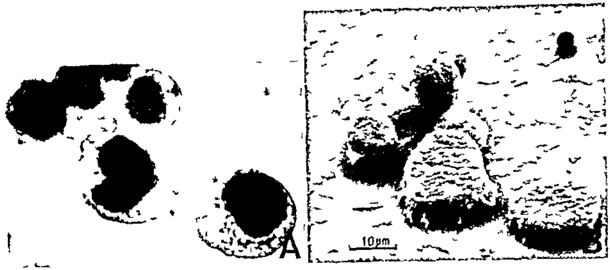

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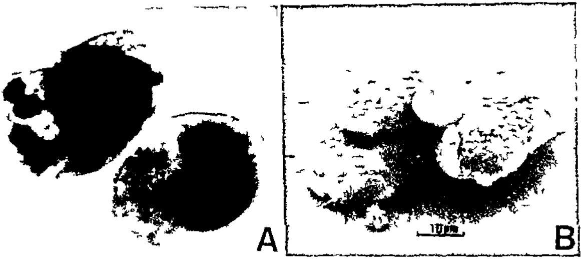

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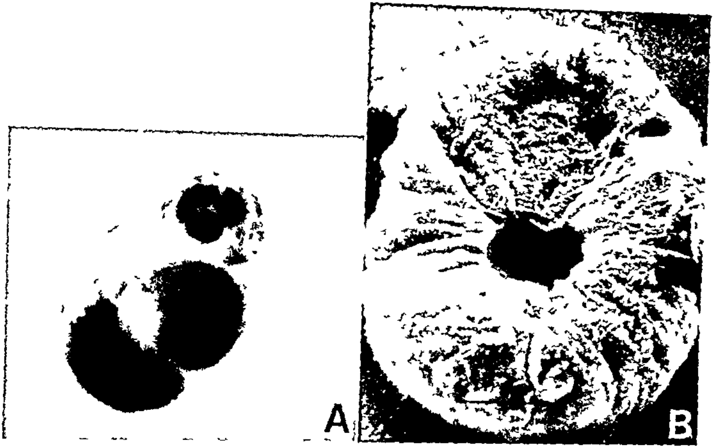

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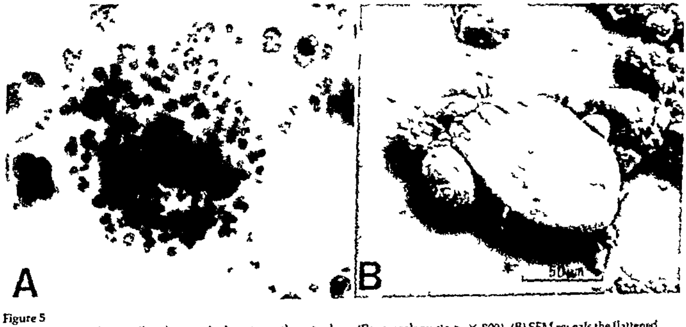

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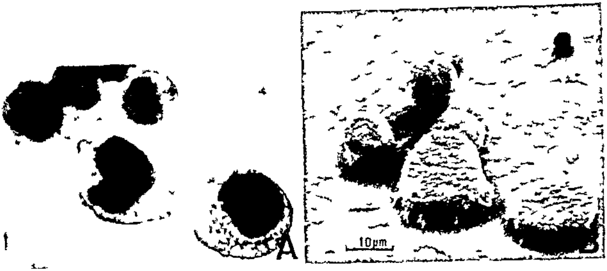

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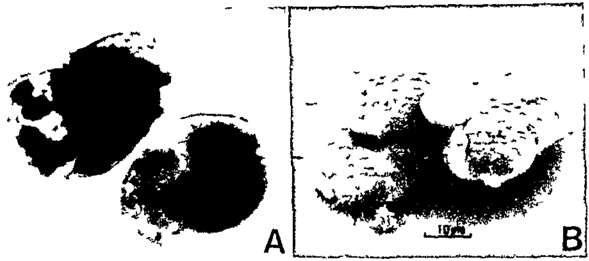

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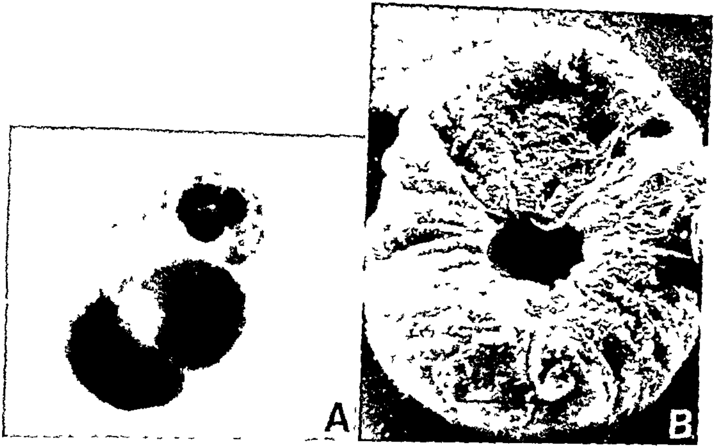

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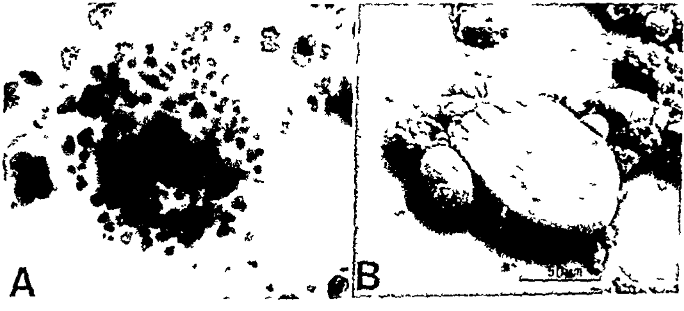

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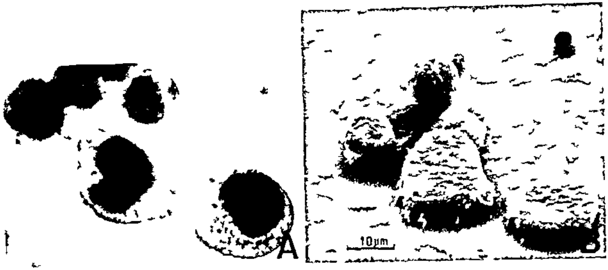

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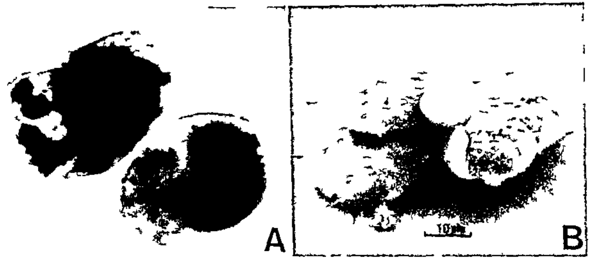

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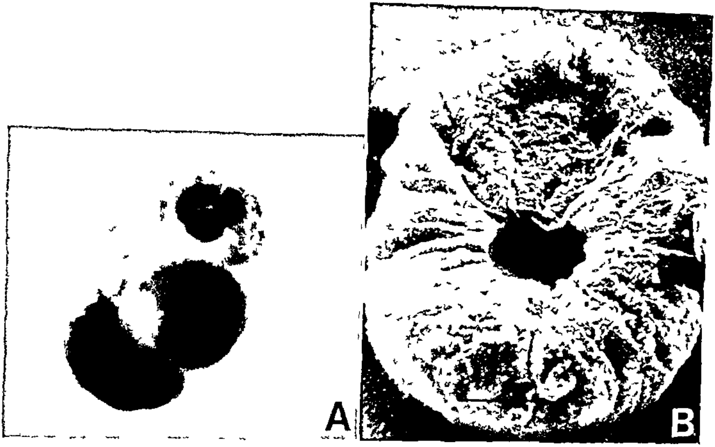

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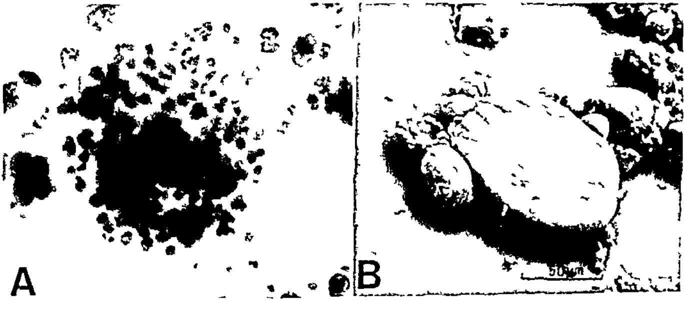

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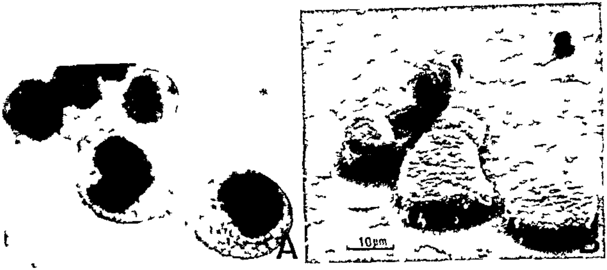

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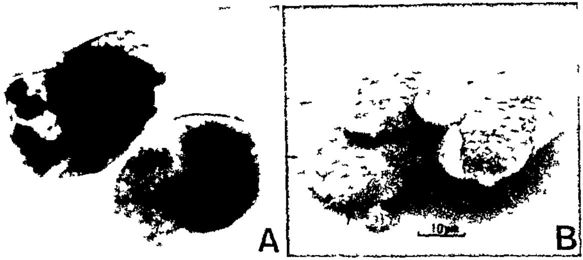

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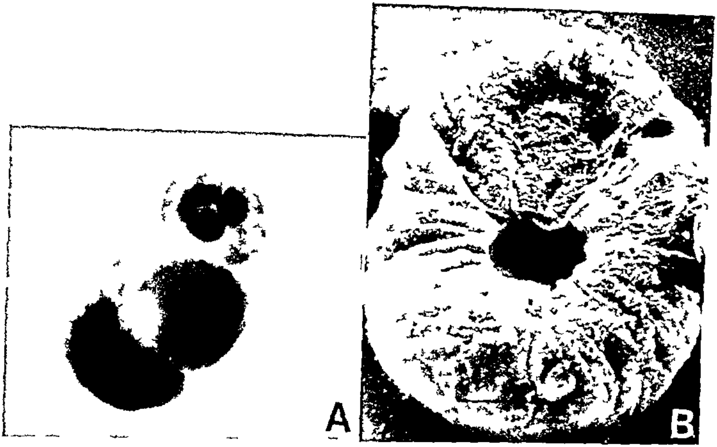

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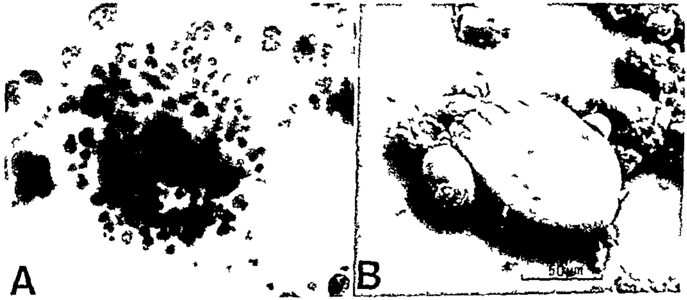

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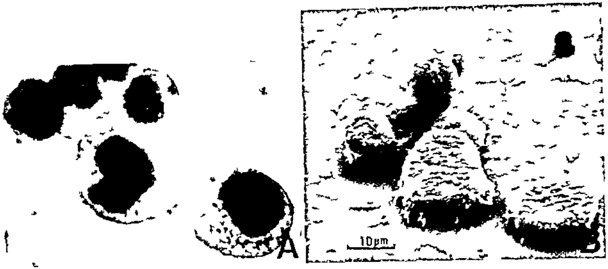

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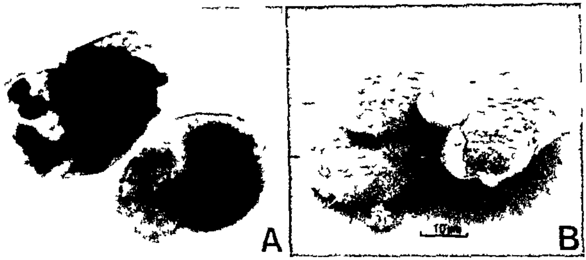

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7 Nash AD Stout AP Giant cell carcinoma of lung Cancer 11 369 3 6 1958

8 Ozzello L Stout AP The epithelial origin of giant cell carcinoma confirmed by tissue culture Cancer 14 1052 1056 1961

9 Razzuk MA Lynn JA Kingsley WB Race GJ Urschel HC Jr Paulson DL Giant cell carcinoma of the lung J Thorac Cardiovasc Surg 59 574 580 1970

10 Razzuk MA Urschel HC Jr Albers JE Martin JA Paulson DL Pulmonary giant cell carcinoma Ann Thorac Surg 21 540 545 1976

11 Takenaga A Matsuda M Horai T Ikegami H Hattori S Scanning electron microscopy in the study of lung cancer New technique of comparative studies on the same lung cancer cells by light microscopy and scanning electron microscopy Acta Cytol 21 90-95 1977

12 Tateishi R Matsuda M Hattori S Electron microscopic studies on giant cell carcinoma of the lung with reference to its histogenesis Annu Rep Center for Adult Dis 10 11 22 1970

Indian Academy of Cytologists

The Ninth Annual Meeting of the Indian Academy of Cytologists was held November 10 and 11 1979 at Baroda The scientific program of the meeting consisted of a panel discussion seminar a diagnostic cytology seminar and proffered paper sessions The panel discussion was on cytology as a discipline in undergraduate and post graduate education continuing education in cytology

The Academy Oration for the year 1979 was delivered by Dr K D Virkar Review of Vaginocervical Cytology with Exogenous Estrogens and Progestin Thirty three selected papers were presented at the proffered paper sessions

The guest lecture for the year was delivered by Dr Erica Wachtel (UK) Scope of Endocrine Cytology

The Indian Academy of Cytologists appointed a three member standing committee in 1975 consisting of Dr Usha K Luthra (Chairperson) Dr Meera Aikat and Dr S Monga The main functions of the Committee for Accreditation and Examination are (1) to arrange for accreditation of cytology laboratories in the country and (2) to organize an examination for cytotechnicians and cytotechnologists The tenure of the committee initially was three years but was extended for a further period of three years The following laboratories have been approved by the committee for accreditation for diagnostic service in cytology in the country so far

1978

1 Cytology Laboratory Sir Ganga Ram Hospital New Delhi (under Prof P N Wahi F I A C (hon))
2 Cytology Laboratory at P G I Chandigarh (under Prof Meera Aikat M I A C)
3 Baroda Cancer Detection Centre Baroda (under Dr S Amin)

1979

4 Tata Memorial Centre Bombay (under Dr J Jussawalla)
5 Cytology Laboratory A I I M S New Delhi (under Dr Kusum Verma)
6 Cytology Research Centre New Delhi (under Prof Usha K Luthra F I A C)

Identification of Crystals in Joint Fluids

Paul Zaharopoulos M D M I A C
Jick Yee Wong M S M T , C T (ASCP)

The inflammatory process present in synovial arthritic specimens can be diagnosed by crystal type identification performed in cytopathology laboratories by the use of simple methods These methods involve wet coverslipped preparations of fresh fluid specimens or sediments and compensated polarizing microscopy which can be performed with a routine microscope equipped with polarizing filters and a substitute compensator Details of the methodology are described The morphologic characteristics of the crystals are described in cases of gout pseudogout and arthritis of other types in which secondary calcifications are present

The various forms of synovial fluid cytology have long been described and since most cases with accumulation of fluid in joints are of an inflammatory nature these specimens are customarily and adequately examined by laboratories of well-organized rheumatology services However cytology laboratories are routinely involved in the examination of these fluids and are asked to provide diagnostic information regarding the type of pathologic process present In many cases routine cytopreparations stained by the Papanicolaou method seem to be adequate However when the inflammatory process is associated with deposition of crystals in the joint tissues as in the crystal induced types of synovitis gout and pseudogout the identification of these crystals in routinely fixed and stained specimens is difficult It is the purpose of this paper to discuss simple methods of overcoming this difficulty and to describe certain basic morphologic features of such specimens

Materials and Methods

Nineteen fresh synovial fluid specimens from joints deriving from 18 cases of arthritis were examined They included two cases of gout (one primary and one secondary following radiation for breast carcinoma with extensive bone metastasis) four cases of pseudogout and 12 cases of other types of arthritis namely four cases of septic arthritis (three of the staphylococcal and one of the gonococcal type) three cases of osteoarthritis one case of rheumatoid arthritis one Charcot joint secondary to systemic Charcot Marie Tooth disease and three cases of post traumatic arthritis The majority of the specimens examined (15 cases) were derived from knee joints one was from the ankle (rheumatoid arthritis) and one from the shoulder joint (Charcot joint)

The specimens were examined for the presence of crystals as soon as they were received This was done by placing a few drops of the specimen on carefully precleaned glass slides wet mounting them sealing the edges with nail polish to prevent rapid dehydration and examining them under a light microscope Lowering the condenser facilitated the identification of crystals by increasing their refractility

If the specimen was hypocellular or the crystals were very scanty precleaned glass slides were prepared by the cytocentrifuge method wet mounted sealed and examined in a similar manner The slides were further subjected to compensated polarizing light microscopy and since there was eventual dete

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Dr Zaharopoulos is Associate Director Cytopathology Laboratory and Faculty Associate Department of Pathology

Mr Wong is Chief Cytotechnologist and Educational Coordinator Cytopathology Laboratory Department of Pathology

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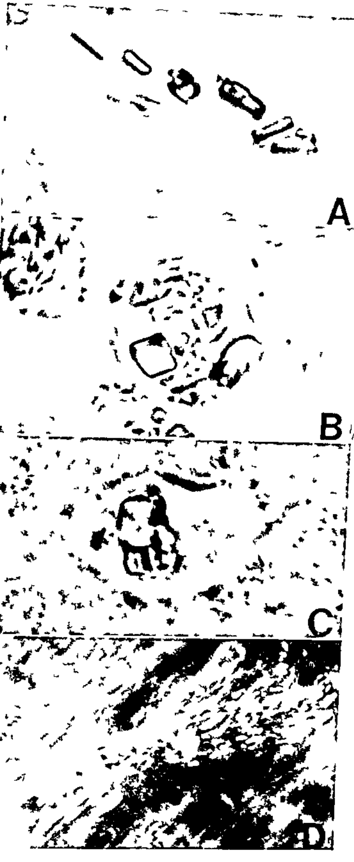
staphylococcal type) In these cases scanty calcific deposits were dispersed free in the synovial fluid specimen or deposited in degenerated cartilagenous tissue fragments (Charcot joint) They appeared mainly as small particles and collections of refractile amorphous looking material and in some instances as larger crystallic particles with concentric calcium deposition rings (Figure 4) On polarizing microscopy they showed either no birefringence or a very weak birefringence with no definite pattern by use of the compensator By their morphologic characteristics and relative paucity in the material they could be easily distinguished from urate and CPPD crystals of gout and pseudogout

Discussion

Gout and pseudogout are the most common types of crystal induced synovitis and their diagnosis is based to a great extent on identification of the respec



re 3
dogout synovial fluid (A) CPPD crystals shaped as rods
nboids and mostly parallelepipids (wet preparation × 2 800)
CPPD crystals within neutrophils (wet preparation × 2 800)
CPPD crystals within a synovial cell (wet preparation ×
0) (D) CPPD crystals providing weak birefringence (positive
se of compensator) (wet preparation polarized microscopy
00)

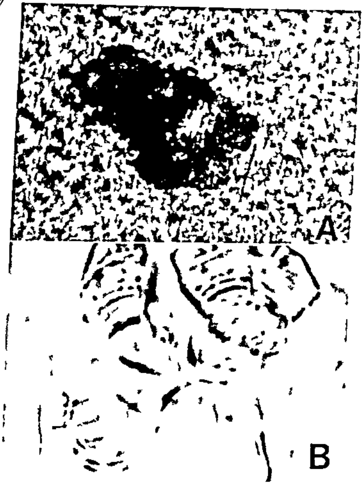


Figure 4
Dystrophic calcifications in synovial fluid (A) Collection of refractile granular crystallic material (wet preparation × 1 120) (B) Crystallic particle with concentric rings (wet preparation × 2 800)

Table I *Cases in Which Crystals in Joint Fluid Specimens Are Present*

Case number	Sex	Age	Joint aspirated	Clinical diagnosis	Crystals on light microscopy	Birefringence on compensated polarizing microscopy	Cytologic diagnos
1	M	57	Knee	Gout (primary)	Consistent with MSU crystals	Strong negative birefringence	Gout (primary)
2	M	76	Knee	Metastatic malignancy vs degenerative arthropathy	Consistent with MSU crystals	Strong negative birefringence	Gout (secondary)
3	M	62	Knee	Suspected pseudogout	Consistent with CPPD crystals	Weak positive birefringence	Pseudogout
4	F	64	Knee	Noninfectious arthritis	Consistent with CPPD crystals	Weak positive birefringence	Pseudogout
5	M	76	Knees bilateral	Osteoarthritis with effusion	Consistent with CPPD crystals	Weak positive birefringence	Pseudogout
6	M	84	Knee	Suspected pseudogout	Consistent with CPPD crystals	Weak positive birefringence	Pseudogout
7	M	63	Shoulder	Charcot joint	Consistent with dystrophic calcifications	Birefringence weak variable	Confirms clinical
8	F	36	Knee	Posttraumatic arthritis with osteomalacia of patella	Consistent with dystrophic calcifications	No birefringence	Confirms clinical
9	M	43	Knee	Posttraumatic	Consistent with dystrophic calcifications	No birefringence	Confirms clinical
10	F	69	Knee	Suspected septic arthritis	Consistent with dystrophic calcifications	Birefringence weak not determinable	Confirms clinical (staphylococcal)

tive crystals in synovial fluid specimens This can be performed in the cytopathology laboratory by use of simplified methods as already described

Gout is characterized by hyperuricemia and deposition of monosodium urate crystals in and about the joints It is primary related to an autosomal dominant genetically determined defective metabolism of uric acid and secondary in which hyperuricemia results from a direct increase of nucleic acid catabolism

Pseudogout reflects an abnormality in inorganic pyrophosphate metabolism of familial occurrence transmitted as an autosomal recessive character [3] and results in deposition of calcium pyrophosphate dihydrate crystals in the matrix of fibrocartilagenous structures of major joints [4] This results in cartilage degeneration and rupture into the synovial space provoking the acute attack

Clinically and radiologically the two diseases may share considerable similarities and cases of their coexistence with the presence of both respective varieties of crystals in the same joint have been described [1,4] Therefore although the MSU and C
crystals are tentatively identifiable by their ligh
croscopic features alone the use of compensate
larized microscopy is necessary for their acc
characterization

Secondary calcifications of the dystrophic typ
occur in diseases of the joints in which there is d
eration and destruction of tissues These deposi
possibly of the calcium hydroxyapatite type as
cated by extensive study by McCarty et al[4] of
ver knee menisci exposed to trauma In our
they could be readily distinguished from MS
CPPD crystals because they did not conform t
MSU and CPPD specific morphologic characte
and type of birefringence

Other unusual types of crystals in synovial
have also been described They include chole
plate like crystals with a notched corner in rh
toid disease corticosteroid rod and granule-like
tals with variable patterns of birefringence foll
intraarticular injection of these drugs and intr
plasmic oxalate crystals in leukocytes after erro

use of oxalate as synovial fluid anticoagulant [2 10] *Such* occurrences although infrequent should also be kept in mind when examining synovial fluid specimens for crystals

Acknowledgments

We express our appreciation to the staff of the UTMB Pathology Photography Laboratory under the direction of Lew Webb L H D for their kind assistance in preparing and presenting this study

References

1 Good AE Rapp R Chondrocalcinosis of the knee with *gout rheumatoid* arthritis N Engl J Med 277 286-290 1967

2 Katz WA Rheumatic Diseases *Diagnosis and Management* Philadelphia JB Lippincott 1977, 284 288 697 727

3 *McCarty DJ Jr Gatter RA* Brill JM Hogan JM Crystal deposition diseases sodium urate *(gout) and calcium* pyrophosphate (chondrocalcinosis pseudogout) JAMA 193 129-132 1965

4 McCarty DJ Jr Hogan JM Gatter RA Grossman M Studies *on* pathological calcifications in human cartilate I Prevalence *and types of crystal deposits in the menisci of two hundred fifteen* cadavera Bone Joint Surg 48A *309 325 1966*

5 *McCarty DJ Jr* Silcox DC Gout and pseudogout Geriatrics *28 110-120 1973*

6 Moskowitz RW Katz D Chondrocalcinosis coincidental to other rheumatic *diseases Arch Intern Med 115 680-683* 1965

7 *Owen DS Jr* A cheap and useful compensated polarizing microscope New Engl J Med 285 1152 1971

8 Phelps P Steele AD McCarty DJ Jr Compensated polarized light microscopy Identification of crystals in synovial fluids from gout and pseudogout *JAMA 203 508-512* 1968

9 Reginato AJ Schumacher HR Martinez VA The articular cartilage in familial chondrocalcinosis Light and electron microscopic study Arthritis Rheum 17 977-982 1974

10 Schumacher HR Intracellular crystals in synovial fluids anticoagulated with oxalate N Engl *J Med 274 1372 1374 1966*

Fifth International Symposium on Flow Cytometry Clinical and Biological Applications

The Fifth International Symposium on Flow Cytometry Clinical and Biological Applications will be held at the Orsini-Odescalchi Castle Bracciano Rome Italy December 2-5 1980 under the sponsorship of the Society for Analytical Cytology The Program Committee consists of F *Mauro (Rome) O D Laerum* (Bergen) C Nervi (Rome) J Schumann (Munster) and M A Van Dilla (Livermore) For further information write to Symposium on Flow Cytometry c/o I M eR S Via L Bodio 58 00191 Rome Italy

Copper Deposits in Fine Needle Aspiration Biopsies in Primary Biliary Cirrhosis

Pentti Sipponen M D
Pekka Pikkarainen M D
Erkki Vuori M D
Mikko Salaspuro M D

The occurrence of copper deposits in liver cells obtained by fine needle aspiration biopsy from the livers of 21 patients with primary biliary cirrhosis (PBC) was studied Eight (38%) of these 21 patients showed perinuclear rubeanic acid positive deposits in fine needle specimens which were alcohol fixed and prepared using a filter technique The density and frequency of the deposits increased with advancing hepatic injury and with increasing liver copper concentration The study suggested that hepatic copper deposits in advanced chronic cholestatic liver diseases such as PBC can easily be demonstrated by the simple and safe fine needle aspiration liver biopsy technique

Wilson s disease is a classic example of disturbed metabolism and increased hepatic accumulation of copper [2,20] Equally large accumulations of copper are also seen in primary biliary cirrhosis (PBC) in other chronic cholestatic liver diseases[1,6,13,19] and in Indian childhood cirrhosis [6] These copper accumulations can easily be demonstrated as coarse perinuclear deposits in histologic liver sections by using copper stains or Shikata s orcein method [5,11,21] The presence of these deposits has suggestive diagnostic significance and furthermore gives prognostic information in chronic active hepatitis [12]

Fine needle aspiration biopsy of the liver is a safe diagnostic procedure[1,7] and can be carried out when other biopsies are contraindicated In order to clarify whether copper deposits could be demonstrated in fine needle cytology we performed histochemical copper and orcein stains on 21 fine needle biopsies from patients with PBC

Materials and Methods

Patient Series and Biopsy Specimens

The series consisted of 21 patients with primary biliary cirrhosis (PBC) The diagnosis was based on accepted clinical biochemical immunologic and histologic criteria of the disease [14] as published in detail before [12] One percutaneous fine needle aspiration liver biopsy and two forceps biopsies obtained at laparoscopy were obtained from each patient Fine needle biopsies were fixed overnight in 50% alcohol filtered on a Millipore membrane and stained The first of the two forceps biopsies was fixed in 10% neutral-buffered formalin embedded in paraffin and sectioned

In all cases the histologic stage of the PBC was determined from the normally stained histologic sections and graded (I to IV) according to Sherlock and Scheuer [14]

Staining Methods

The same methods and staining procedures were used for both the cytologic and histologic specimens Copper was stained with rubeanic acid [8] Orcein staining was performed as described by Shikata et al [11]

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Copper deposits in histologic sections were scored into four groups (− to +++) according to the density and amount of copper deposits and without knowledge of the liver copper concentration The findings were later related to the absolute copper concentration of the liver

Determination of the Hepatic Copper Concentration

The second concocotomy biopsy was used for the chemical analysis of the liver copper Specimens were dry ashed and the copper concentration was determined by atomic absorption spectrophotometry after dissolution in hydrochloric acid The accuracy of the method was tested by using the Standard Reference Material no 1577 bovine liver produced by the National Bureau of Standards (NBS) Washington DC [21]

Results

Copper deposits were found in eight (38%) of 21 fine needle cytology specimens (Table I) Eighteen (86%) of these 21 patients showed copper and/or copper protein complexes (rubeanic acid and/or orcein positivity respectively) in histologic liver sections In fine needle specimens copper deposits were stained only by the rubeanic acid method but not with orcein Copper deposits in these cytologic specimens were coarse green brown perinuclear deposits and were morphologically similar to the rubeanic acid-positive and orcein positive deposits of the formalin-fixed biopsy specimens (Figures 1 and 2)

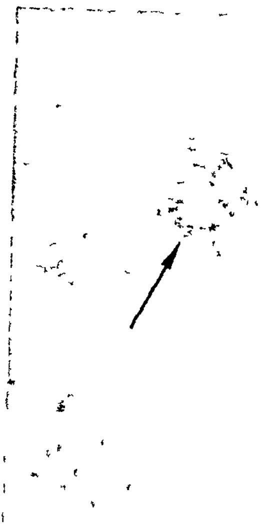

Figure 1

Rubeanic acid positive copper deposits (arrows) in the perinuclear area of liver cells taken by fine needle aspiration biopsy (rubeanic acid staining × 550)

The presence of copper deposits in fine needle biopsies correlated positively with the absolute copper concentration of the liver (Figure 3) and also with the scored copper quantities of the histologic liver sections (Table I) The mean absolute liver copper concentration of the rubeanic acid positive group was 514 ± 225μg/gm of dry weight liver tissue (range 98μg/gm to 735μg/gm) and that of the negative patients was 265 ± 157μg/gm (range 85μg/gm to 515μg/gm)

Table I *Correlation Between Copper Deposits in Histologic Liver Sections and in Fine Needle Aspiration Biopsies in 21 Patients with Primary Biliary Cirrhosis*

	Total number of patients	Density of copper deposits in histologic sections			
		−	+	++	+++
Copper deposits present in fine needle biopsies	8	0	2	3	3
Copper deposits absent in fine needle biopsies	13	3	8	2	0

Copper and copper protein in histologic sections stained with rubeanic acid and orcein − = absent + = some perinuclear deposits ++ = heavy perinuclear deposits in periportal liver cells and +++ = deposits throughout liver lobules

The occurrence of copper deposits in fine needle specimens also correlated positively with the degree of hepatic injury (Table II) Less than one third of the patients with PBC of stages I and II showed copper deposits in aspirated cells but nearly two thirds of patients with stages III and IV showed these deposits

Discussion

This study indicated that liver copper deposits can easily be detected in fine needle aspiration biopsies obtained from patients with primary biliary cirrhosis Although the sensitivity of the method is rather

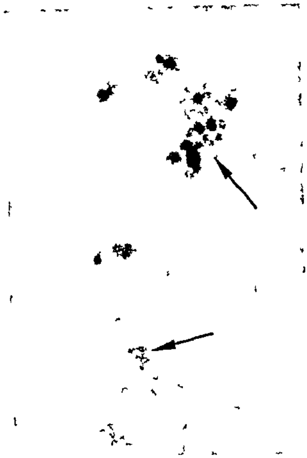

Figure 2
Rubeanic-acid-positive copper deposits (arrows) in the perinuclear area of liver cells in a formalin-fixed paraffin section (rubeanic acid staining × 650)

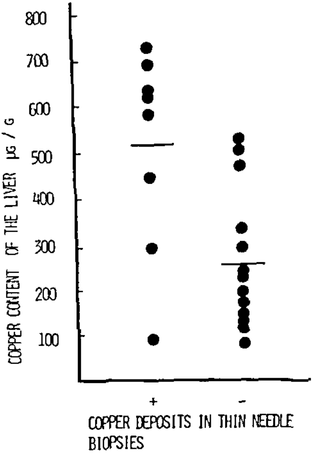

Figure 3
Correlation between copper deposits in fine needle biopsies and copper concentration in the liver Copper content is expressed as µg/gm dry weight

low it does improve with the increasing liver copper concentration associated with more advanced hepatic injury Eight (44%) of 18 patients with demonstrable copper deposits in histologic sections also showed copper deposits in fine needle cytologic specimens On the other hand six (75%) of eight patients with heavy copper deposits (grades + + and + + + in normal histology) had copper deposits in fine needle cytology The absence of copper deposits in fine needle aspiration biopsies from the patients with low liver copper concentrations was probably due to the differences in hepatic copper distribution In early stages of PBC copper deposits are focal and are therefore only occasionally biopsied [17]

Excess hepatic copper in the patients with PBC appears to be in the form of copper protein complexes We have suggested that the protein moiety of this complex can be stained with orcein [11,1] Orcein stain

Table II *Correlation Between the Stage of Primary Biliary Cirrhosis (PBC) and Copper Deposits in Fine Needle Aspiration Biopsies*

	Total number of patients	Stage of PBC			
		I	II	III	IV
Copper deposits present in fine needle biopsies	8	0	[illegible]	4	[illegible]
Copper deposits absent in fine needle biopsies	13	4	5	3	1

ing of copper associated protein in formalin fixed histologic specimens is more sensitive than the staining of copper itself with either rhodanine or rubeanic acid However our data indicate that orcein staining cannot be used in cytologic specimens Presum ably the longer alcohol fixation of the cytologic samples destroys the staining property of the protein moiety

The presence of copper deposits in liver suggests certain diagnostic possibilities[13,17,18] dense deposits indicate PBC or closely related chronic cholestatic liver diseases There is also evidence that copper deposits in chronic active hepatitis (CAH) have prognostic significance [12]

In our previous material we could find orcein-positive copper-protein deposits in about 20% of cases with HB_sAg negative CAH These patients did not respond at all or had a poor response to the immunosuppressive treatment with cortisone and/or azathioprine in contrast to a good response in patients without copper deposits [12] The deposits however are more scanty in CAH than in typical PBC [17] which may limit the differential use of fine needle aspiration biopsy in CAH

Hepatic copper accumulations in Wilson s disease have been known for several decades However there are some differences in the subcellular distribution of copper in Wilson s disease and in chronic cholestatic liver disorders [5] Furthermore biliary copper excretion in PBC is not decreased as it is in Wilson s disease [10] suggesting different pathogenetic mechanisms for hepatic copper accumulations Nevertheless in Wilson s disease copper deposits can also be demonstrated in histologic sections with copper stains such as rubeanic acid [20] Therefore it is reasonable to believe that these deposits could also be demonstrated by the fine needle aspiration technique

A recent paper by Portman et al[9] has described heavy hepatic copper-protein deposits in most patients with Indian childhood cirrhosis (ICC) It is probable that the demonstration of copper in fine needle biopsies could be a simple and safe method of indicative demonstration of this disease in endemic areas of ICC

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References

1 Carney CN Clinical cytology of the liver Acta Cytol 19 244 250 1975

2 Cumings JN The copper and iron content of brain and liver in the normal and in hepato-lenticular degeneration Brain 71 401-415 1948

3 Fleming CR Dickson ER Baggenstoss AH McCall JT Copper and primary biliary cirrhosis Gastroenterology 67 1182 1187 1974

4 Hunt AH Parr RM Taylor DM Trott NG Relation between cirrhosis and trace metal content of liver Br Med J 2 1498-1501 1963

5 Jain S Scheuer PJ Acher B Newman SP Sherlock S Histological demonstration of copper and copper-associated protein in chronic liver diseases J Clin Pathol 31 784 790 1978

6 Leeson PM Fourman P A disorder of copper metabolism treated with penicillamine in a patient with primary biliary cirrhosis and renal tubular acidosis Am J Med 43 620-635 1967

⑦ Lundqvist A Fine needle aspiration biopsy of the liver Applications in clinical diagnosis and investigation Acta Med Scand [Suppl] 520 1 28 1971

8 Pearse AGE Histochemistry Theoretical and Applied Third edition Second volume London J&A Churchill 1972 p 1411

9 Portman M Tanner MS Mowat AP Williams R Orcein positive liver deposits in Indian childhood cirrhosis Lancet 1 1338 1340 1970

10 Salaspuro M Pikkarainen P Sipponen P Vuori E Miettinen T Hepatic copper in primary biliary cirrhosis Biliary secretion and response to penicillamine treatment Gastroenterology 74 1161 1978

11 Salaspuro M Sipponen P Demonstration of an intracellular copper-binding protein by orcein-staining in long-standing cholestatic liver diseases Gut 17 787 790 1976

12 Salaspuro MP Sipponen P Ikkala E Kolho L Makkonen H Miettinen T Rasanen J Siurala M Clinical correlations and significance of orcein positivity in chronic active hepatitis and primary biliary cirrhosis Ann Clin Res 8 206-215 1976

13 Salaspuro MP Sipponen P Makkonen H The occurrence of orcein positive hepatocellular material in various liver diseases Scand J Gastroenterol 11 677-681 1976

14 Sherlock S Scheuer PJ The presentation of 100 patients with primary biliary cirrhosis N Engl J Med 289 674-678 1973

15 Shikata T Uzawa T Yoshiwara N Akatsuka T Yamazaki S Staining methods of Australia antigen in paraffin section Detection of cytoplasmic inclusion bodies Jpn J Exp Med 44 25-36 1974

16 Sipponen P Orcein positive hepatocellular material in long standing biliary diseases I Histochemical characteristics Scand J Gastroenterol 11 545-552 1976

17 Sipponen P Salaspuro MP Makkonen H Histological characteristics of chronic hepatides and primary biliary cirrhosis with special reference to orcein-positive hepatocellular accumulations Ann Clin Res 8 200-205 1976

18 Sipponen P Salaspuro MP Makkonen H Orcein positive hepatocellular material in histological diagnosis of primary biliary cirrhosis Ann Clin Res 7 273 277 1975

19 Smallwood RA Williams HA Rosenoer VM Sherlock S Liver-copper levels in liver disease Studies using neutron activation analysis Lancet 2 1310 1313 1968

20 Sternlieb I Evolution of the hepatic lesion in Wilson s disease (hepatolenticular degeneration) Prog Liver Dis 4 511 525 1972

21 Vuori E Huunan Seppala A Kilpio JO Biologically active metals in human tissues I The effect of age and sex on the concentration of copper in aorta heart kidney liver lung pancreas and skeletal muscle Scand J Work Environ Health 4 167 175 1978

Deutsche Gesellschaft fur Zytologie

Auf der Mitgliederversammlung der Deutschen Gesellschaft fur Zytologie am 7 November 1979 in Munchen wurde der Gesamtvorstand fur die Jahre 1980/81 neu gewahlt Der Vorstand setzt sich wie folgt zusammen

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Vaginal and Urinary Cytology and Blood Hormonal Examinations in Patients with Premature Rupture of Membranes

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Haim Yaffe M D
Shalom Segal, Ph D

Vaginal smears and urinary sediments of 29 pregnant women in their third trimester with premature rupture of membranes (PRM) were examined Plasma radioimmunoassays (RIA) for estriol human chorionic gonadotropin human placental lactogen and progesterone were performed Twenty three normal pregnant women in the third trimester serving a controls underwent the same examinations Th vaginal smears and urinary sediments were evaluate as reflecting disturbed slightly disturbed and norma placental function Good correlation was found between the vaginal smears showing the typical an suggestive aspects of PRM and the placental functio reflected in the same smears and the respectiv urinary sediments From the hormonal examination performed the plasma RIA for progesterone presented significantly lower results in the PRM cases than in the control group The results obtained suggest a lowering of the placental function in cases with PRM as reflected in cytologic and hormonal examinations

In a previous study a typical cytologic feature was described in vaginal smears of gravid patients with obvious premature rupture of membrane (PRM) [1] In two thirds of such patients the vaginal cytology was characterized by the presence of sheets and separated cells loss of tinctorial affinity of nuclei and cytoplasm and reduction of the bacterial flora This cytologic aspect suggested an underlying hormonal disturbance but the mechanical washing of the vaginal walls by the amniotic fluid also could have contributed to it

In order to determine the possible role of this mechanical factor the urinary sediments have been studied in parallel with the vaginal cytology in cases of premature rupture of membranes In these cases a correlation was found between the vaginal and urinary cytology with both types of smears reveal ing a hormonal disturbance [2] In order to further substantiate this finding estriol human placental lactogen (HPL) human chorionic gonadotropin (HCG) and progesterone levels were determined by plasma radioimmunoassays (RIA)

Materials and Methods

Twenty nine patients in the third trimester of pregnancy with an obvious escape of amniotic fluid from the vagina were studied Regular uterine contractions did not begin within six hours of the rupture of membranes in any of these patients There were six patients between the 29th and 36th weeks of pregnancy and 23 between the 37th and 40th weeks Twenty three women in the third trimester of normally developing pregnancy served as the control group Five were between the 30th and 36th weeks and 18 between the 37th and 40th weeks of pregnancy

Vaginal smears were obtained from the upper third

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of the lateral vaginal wall They were fixed with polyethylene glycol and stained by the Papanicolaou method The urinary sediments were prepared by centrifugation (2 000 rpm for five minutes) of fresh specimens of urine twice washed with normal saline and air dried [7] The staining was done by the Papanicolaou method Plasma estriol HCG HPL and progesterone levels were determined utilizing radioimmunoassay kits for these hormones purchased from C I S (Gif-sur Yvette France) The blood samples were obtained before the beginning of the contractions

Statistical analysis was done by the Student t and Mann Whitney tests

Evaluation of the Smears

Vaginal smears were evaluated with regard to the cytologic features described in cases of PRM and were classified as typical suggestive of this condition or normal Smears were considered typical of PRM when three criteria were observed (1) the presence of cells either separately or as sheets with clumping extremely rare (2) washed-out nuclei and cytoplasm and (3) diminution or absence of Doderlein bacilli (Figure 1) Smears showing these criteria to a lesser degree and which thus did not fit into either the typical or the normal category were considered suggestive of PRM In addition vaginal and urinary smears were evaluated for placental function and classified into one of three categories disturbed slightly disturbed or normal Disturbed smears were those which presented the following three criteria (1) dispersion of clumps and a tendency toward separation of cells (2) flatness of cells and (3) the presence of superficial squamous and/or parabasal cells (Figures 1 and 2) When these criteria were present to a slight degree the smears were classified as indicating slightly disturbed placental function

The different aspects of the PRM condition were correlated with the features reflecting placental func

Figure 1
Case [illegible] Vaginal smear presenting [illegible] characteristic of PRM sheets of cells with washed-out cytoplasm and absence of Doderlein bacilli [illegible] indicating disturbed placental function are also present dispersion of clumps flatness of cells and superficial squamous [illegible]

Figure 2
Case [illegible] Urinary sediment smear characteristic of disturbed placental function Note separation and flatness of [illegible] intermediate and parabasal cells (× [illegible])

Table I *Relationship Between Vaginal Cytologic Pictures Associated with Premature Rupture of Membranes (PRM) and Vaginal Smears and Urinary Sediments Indicating Placental Function*

		Cytology placental function						
		Vaginal smears			Urinary sediments			
Vaginal cytologic picture with PRM	Total no of cases	*Disturbed*	*Slightly Disturbed*	*Normal*	Disturbed	Slightly Disturbed	Normal	Unsatisfactory
Typical	16	11	5	—	7	7	—	—
Suggestive	12	—	12	—	—	8	1	3
Normal	1	—	—	—	—	—	2	—

Figures indicate number of cases

Table II *RIA Determinations of Plasma Estriol HCG HPL and Progesterone in Patients with Premature Rupture of Membranes (PRM) Showing the Typical and Suggestive Cytologic Aspects of This Condition as Compared with Those of Normal Pregnant Women*

Vaginal cytology	No of cases	Estriol		HCG		*HPL*		*Progesterone*	
		Mean	SE	*Mean*	*SE*	Mean	SE	Mean	SE
Typical of PRM	*11*	*11 8*	*2 8*	*16 690*	*4 396*	6 18	0 70	54 2	7 04
Suggestive of PRM	*17*	*13 8*	*1 71*	*12 678*	*3 050*	6 80	0 30	72 2	8 32
Normal pregnant	23	29 1	7 4	*25 950*	*5 160*	6 28	*0 39*	*126 4*	4 7

SE = standard error
Highly significant difference with regard to normal controls (p < 0 001)

tion observed in the same smears and in the urinary sediment smears

Results

The results are presented in Table I Two thirds of the cases with typical aspects of PRM showed evidence of disturbed placental function according to the *vaginal smears* The urinary sediments on the other hand revealed disturbed placental function in only half of these cases while slightly disturbed placental function was observed in the other half There was a strong correlation between the vaginal smears suggestive of PRM and the vaginal and urinary cytology which reflected the slightly disturbed placental function

The mean and standard errors of plasma RIAs for estriol HCG HPL and progesterone from the cases with smears revealing the cytologic aspects typical and suggestive of PRM as compared with those of normal pregnant women are presented in Table II Highly significant differences in the progesterone levels between the PRM cases and the normal controls (p < 0 001) have been found The difference was significant for both the Student t and the Mann Whitney tests The spread of the values for this hormone in the PRM cases and normal pregnancies as related to the week of gestation is given in Figure 3

Discussion

The results of this study suggest that in cases of PRM with obvious escape of amniotic fluid there is an *underlying hormonal disturbance* as evidenced by vaginal and urinary cytology and by plasma levels of progesterone as determined by RIA The etiology of this condition is not known and many hypotheses have been advanced for its elucidation The diagnosis of PRM is very often difficult to establish which explains the large number of tests used for this purpose On the other hand cases with obvious escape of amniotic fluid from the vagina present good evidence for this condition *It has been shown that in* these cases there is a characteristic cytologic picture which can be used for diagnostic purposes [3] The vaginal smears of the PRM cases revealed in addition to these characteristic features other criteria which indicated an underlying hormonal disturbance In order to exclude the effect mechanical washing by the amniotic fluid might have on the cytologic aspects urinary sediments were studied in parallel in cases with PRM Good correlation was found between the vaginal and urinary smears suggesting an underlying hormonal disturbance in cases of PRM [2]

In order to further substantiate this finding this investigation measured plasma levels of estriol HPL HCG and progesterone by RIA as well as by studying

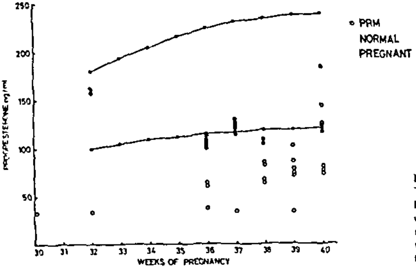

Figure 3
The spread of plasma progesterone RIA values of PRM and normal pregnant women related to the week of gestation. The two solid lines represent normal ranges for plasma RIA progesterone by week of pregnancy as indicated by the manufacturer.

vaginal and urinary cytology.

The results obtained confirmed again the correlation between the vaginal and urinary smears in cases of PRM indicating a disturbed hormonal placental function. Plasma RIA measurements of progesterone were diminished to a degree highly significant in relation to the controls (Table II, Figure 3). These hormonal results thus strengthen the cytologic findings, both vaginal and urinary, reflecting a hormonal disturbance in cases with PRM.

It is to be noted that among the many hypotheses advanced to explain the etiology of PRM, not one related to a hormonal cause has been reported, to the best of our knowledge. Little information referring to the hormonal aspect of PRM has been found in the literature. In their study on the leukocyte alkaline phosphatase (LAP) placental function test, Diamant et al found lower values for this *enzyme in cases of* PRM. In the same study, lower urinary estriol levels in cases of PRM as compared to normal pregnancies were also found. Beischer et al reported a fall in *estriol* values in a case of *PRM*. The presence of possible hormone-dependent disturbances in membrane tensile strength was suggested by Laufer et al in some cases of premature rupture of membranes.

The etiology of PRM can be attributed to a number of factors because of the wide spectrum of circumstances which precede its occurrence.[5] The results of this study, which must be corroborated further, indicate that a hormonal factor may also play a role in the etiology of PRM.

References

1 Beischer NA, Brown JB, MacLeod SC, Smith MZ: The value of urinary oestriol estimation in patients with antepartum vaginal bleeding. J Obstet Gynaecol Br Commonw 74:51-59, 1967

Bercovici B: La cytologie vaginale et urinaire après rupture prématurée des membranes. Arch Anat Cytol Pathol 25:315-317, 1977

3 Bercovici B, Diamant YZ: Vaginal cytology of premature rupture of membranes. Obstet Gynec 137:801-805, 1977

4 Diamant YZ, Sadovsky E, Polishuk WZ: Leukocyte alkaline phosphatase and urinary estriol in the evaluation of placental function. Obstet Gynecol 43:186-190, 1974

5 Gunn GC, Mishell DR, Morton DG: Premature rupture of the fetal membranes. Am J Obstet Gynecol 106:469-483, 1970

6 Laufer A, [illegible] WZ, [illegible] J, Ganzfried R: Studies of amniotic membranes. J Reprod Fertil 11:99-105, 1966

Schaffer B, Wayntraub C: Leucocytogram and estrogen test as a prognostic element in [illegible]. Harefuah 77:18 186, 1969

Double Primary Lung Cancers

With Special Reference to Their Exfoliative Cytology and to the Rare, Malignant "Mixed" Tumor of the Salivary-Gland Type

Yoshio Ebihara, M.D.
Noriko Fukushima, M.D.
Yoko Asakuma, C.M.I.A.C.

Two autopsy cases are reported in which double primary cancers of the lung had been strongly or definitely suspected before death by demonstration of two different types of malignant cells in the sputum as well as in smears of aspirates from pleural fluid and/or mediastinal tumor. By exfoliative cytology, one case was characterized by carcinoma cells of the small-cell type plus the large-cell and/or adenocarcinoma type; the other displayed small-cell type and squamous-cell type malignant cells.

The autopsies definitely revealed in the first case an anaplastic carcinoma of the small-cell type in the left main bronchus and a salivary gland type malignant mixed tumor in the right lower lobe and in the second case an anaplastic carcinoma of the small-cell type in the right upper lobe and a squamous-cell carcinoma in the left upper lobe.

The frequence of occurrence and pathologic diagnosis of double primary lung cancers are reviewed and discussed. A rare type of lung cancer, salivary gland type malignant mixed tumor, is given special reference.

While the discovery on autopsy of double primary cancers developing in different organs is no longer so rare, double primary cancers of the lung are extremely unusual. In particular, it is quite rare or difficult in a patient already suffering from one definite lung cancer to confirm another primary cancer of the same organ before surgery or death. One reason may be that the physician's attention is largely directed to the first cancer, causing a second cancer of the same organ to be overlooked. Another reason may be that the possibility of metastasis of the first cancer cannot be completely ruled out despite suspicion of the presence of the second primary carcinoma.

In the diagnosis of lung cancer, cytologic examination often provides the only information for a definite diagnosis, whether the material is sputa or aspirates taken by chest puncture in very advanced cases, on account of its minimal risks and inconvenience for the patient.• Once the diagnosis of lung cancer has been made, findings in subsequent cytologic examinations contribute more toward indicating the antitumor therapeutic response of the patient than to drawing attention to the possible presence of a second tumor.

The two cases presented below both demonstrated on several occasions two different types of tumor cells in the cytologic examination of sputum and aspirates obtained by thoracentesis or mediastinal centesis, giving a strong suspicion of double cancer of the lung, the presence of which was proved afterwards by autopsy.

Case Reports

Case 1: Male, 72 years of Age

The patient had been told in 1972, at the age of 70, that he had bronchiectasis because of an increased expectoration of phlegm. Since the end of 1974 he had complained of dyspnea and even more sputum, and x-ray examination of the chest at the surgical unit of Fraternity Memorial Hospital revealed an ab

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normally dense shadow in the left lung field and an enlarged opacity of the left hilus The case was diagnosed as lung cancer with metastasis in the left hilar lymph nodes and cobalt irradiation therapy was immediately begun In January 1975 however chest X ray revealed an abnormal shadow located a little to the mediastinal side of the right lung field (Figure 1) The patient s condition gradually deteriorated and he died in March 1975

Cytologic examinations of sputa were positive for malignancy on a total of nine occasions small-cell carcinoma cells on five occasions adenocarcinoma cells on two and large-cell carcinoma cells on two The small-cell carcinoma cells were in no instance found to coexist with any of the other types of tumor cells in the sputum

Gross Pathologic Findings A tumor was located about 3 cm along the mediastinal side of the left main bronchus growing into the bronchial lumen with consequent marked stenosis The neoplastic growth extended continuously into the lower lobe bronchus causing complete obstruction (Figure 2) Consequently the entire lower lobe was collapsed with cylindrical dilatation of the bronchi within this lobe Inasmuch as there were no gross findings of tumor growth into the lung parenchyma the radioopacity noted in the left lower field on the x ray film was considered to be a shadow of parenchymal collapse caused by the carcinoma s growing in the central portion The regional metastatic lymph nodes were fused into a mass measuring about 4 cm in diameter

There was another subpleural nodular tumor mass of 4 cm in diameter in S_6 of the right lower lobe (Figure 3) The pleura of this region was slightly depressed *and the cut surface was grayish white some*what transparent and shiny with a cartilage like firm consistency The tumor was relatively well defined but devoid of encapsulation It was obvious

Figure 1
Case 1 The chest x-ray film shows broadening of the central shadow and a localized lesion (arrow) in the left lower field

Figure 2
Case 1 left lung The tumor growth in the left main bronchus protrudes into the lumen with stenoses extending for 3 cm (arrows) The tumor extends to the lower bronchial mucosa causing complete obstruction and peripheral bronchiectasis (L = metastatic lymph nodes)

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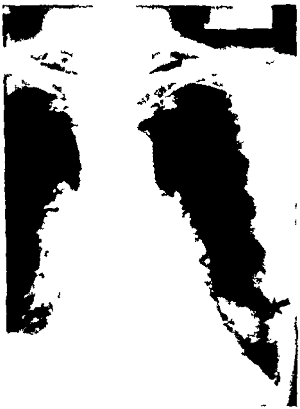

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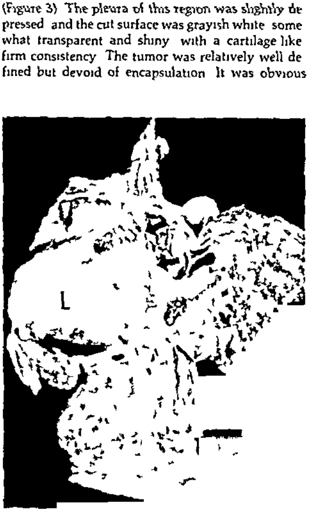

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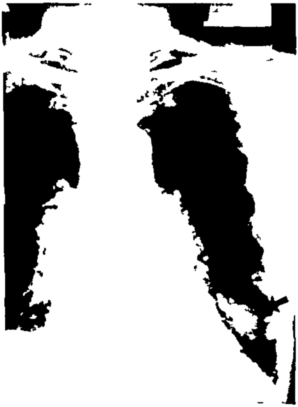

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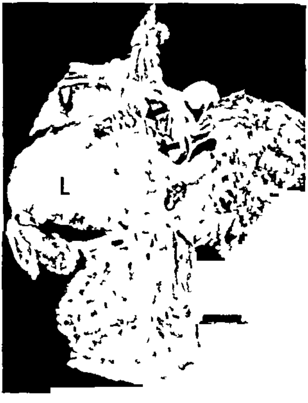

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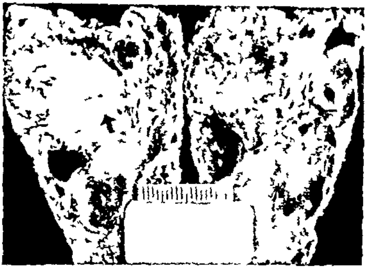


Figure 3
Case 1 The photograph shows two step-sections of another tumor in S of the right lung which includes the involved bronchus B (arrow)

that the central portion of the tumor was continuous with the dilated bronchi There was no evidence of metastasis in lymph nodes within the right lung

Histologic Findings Histologically the tumor extending from the left main bronchus to the left lower bronchus was composed of small spindle-shaped cells possessing round to ellipsoid nuclei which consisted of dense finely granular nucleoplasm (Figure 4) These cells formed solid nests in the tumor mass The tumor was diagnosed as small-cell anaplastic carcinoma fusiform-cell type

The other tumor located in S_4 of the right lung *presented diverse histologic features Most of the tu*mor had a chondroid structure (Figure 5) showing a tendency toward necrosis whereas the remaining via

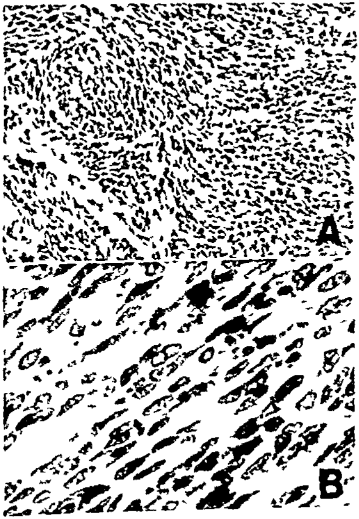


Figure 4
Case 1 (A) *Histology* of the tumor originating in the left main bronchus shows small-cell anaplastic carcinoma spindle-cell type (B) Higher magnification of Figure 4A

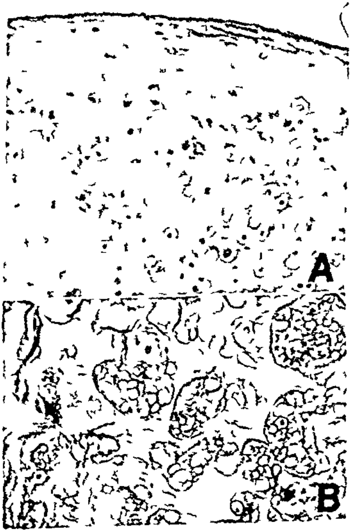


Figure 5
Case 1 second tumor (A) Cartilage-like structure a predominant feature of the second tumor (B) Reticuline staining of the cartilage-like structure makes it clear that the tumor cells consist of alveolar structures

ble portion of the tumor had the following microscopic features Generally a duct lining pattern (Figures 6 and 7) was seen characterized by multiple tubular arrangements of discrete cuboidal cells growing with mutual anastomoses The two-cell thick epithelial elements were fairly characteristic of this structure with the outer cells seeming to be positionally similar to myoepithelial cells They extended into the edematous matrix (Figure 9) where the cells were sporadically interspersed with atypical spindle cells thus presenting the microscopic features of spindle-cell sarcoma (Figure 8) The hydropic clear cell pattern (Figure 9) principally showed a morphologic resemblance to that of the inner cells of the duct lining but was characterized by a markedly diminished stroma This pattern was prominent in the polypoid growths into the bronchial lumen (Figure 9B) The cells in this lesion bore a morphologic similarity to those appearing in the sputum and were recognized as adenocarcinoma cells or large anaplastic carcinoma cells (Figures 10 and 11) Chondroid formation (Figure 5) as described above was observed in most of the tumor This chondroid matrix presented morphologic features of transition from the hyalinous stroma around the tubular structures (Figure 12) and tumor nests were demonstrable by silver impregnations in the chondroid area (Figure 5B) Mucoepidermoid structures (Figure 13A) were seen in the marginal portion of the tumor revealing morphologic features of transition from the tubular structures with squamous metaplasia (Figure 13B) Within very limited areas the stroma was conspicuously edematous thus appearing as myxoma (Figure 14) However tumor cells in these areas were predomi

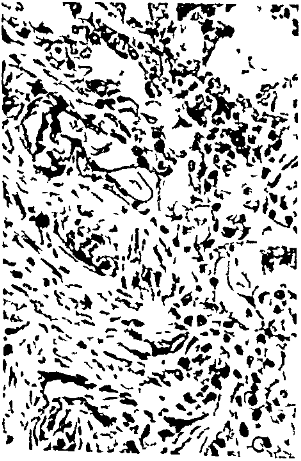


Figure 6
Case 1 second tumor Neoplastic epithelial cells of another tumor in the right lung show a duct lining pattern

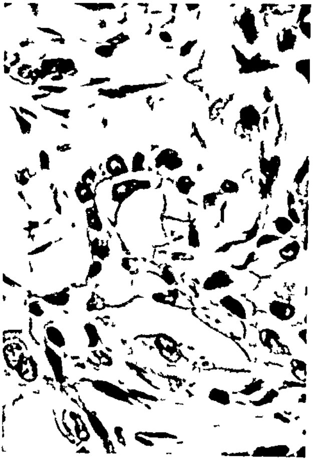
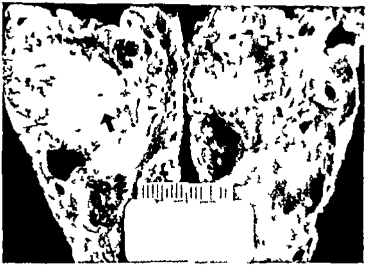


Figure 7
Case 1 second tumor Higher magnification of a part of Figure 6 reveals an atypical gland-like structure covered by a two-cell thick epithelium the outer layer of which seems to be myoepithelial



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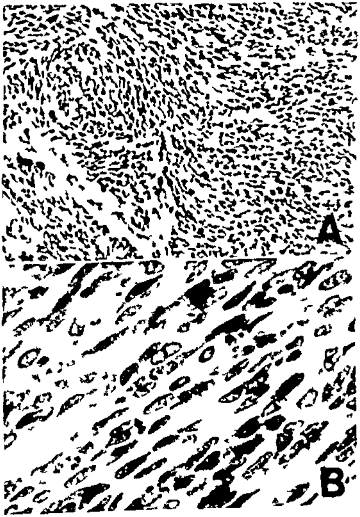

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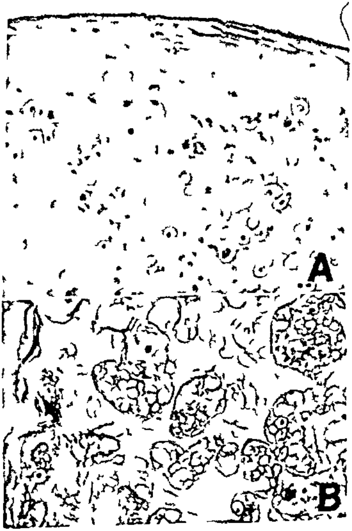

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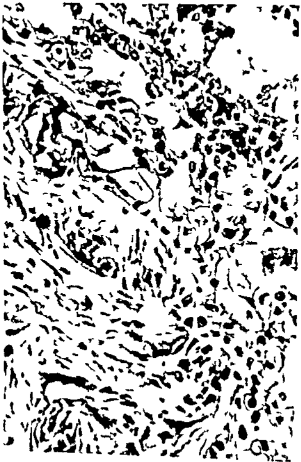

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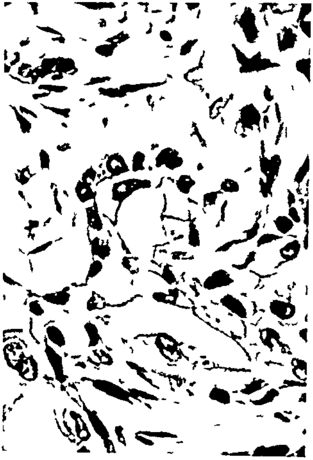

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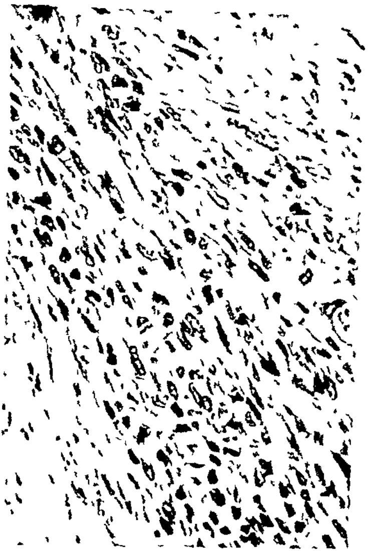


Figure 8
Case 1 second tumor Leiomyosarcomatous lesion

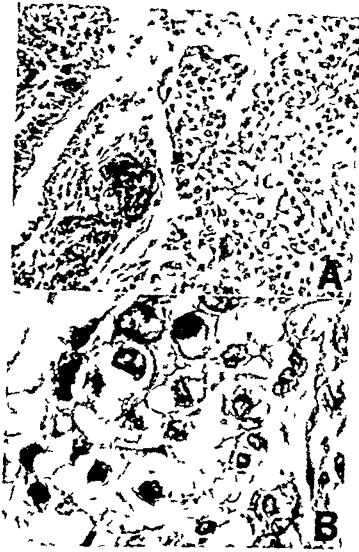


Figure 9
Case 1 second tumor (A) Sheets of large polygonal and vacuolated cells growing into the bronchial lumen (B) Higher magnification of Figure 9A The proliferating cells resemble those seen in the smear material of sputum as shown in Figures 10 and 11

nantly spindle-shaped rather than of stellate form There was no evidence of participation of osteoid or striated muscle cells

As described above the tumor showed a morphologic similarity to a mixed tumor of the salivary gland and accordingly was diagnosed as malignant *mixed tumor* salivary-gland type There was no evidence of distant metastasis of this tumor though it showed invasive growth *in situ*

Cytologic Findings The cells identified as small-cell type carcinoma were polygonal with relatively distinct cellular margins They measured from 8μm to 12μm (mean 9μm) across the greater diameter when observed after Papanicolaou staining (Figure 15) The nucleus measured from 6μm to 12μm (mean 8μm) across the greater diameter and varied widely in profile occasionally showing distinct indentation The nuclear membrane was not thickened the finely granular nucleoplasm was dense and nucleoli were small but prominent These cells often grouped but did not pile up

The cells classified as adenocarcinoma (Figure 11) or large-cell carcinoma (Figure 10) were poorly defined cells measuring 13μm to 34μm (mean 23μm) in their greatest dimension and possessed abundant cytoplasm which had a maximum diameter of 9μm to 17μm (mean 13μm) and was spheroid The cells were either mononuclear or binuclear with the nucleus located somewhat eccentrically The nuclear margin was smooth and not thickened The nucleoplasm was finely granular and characteristically con

Figure 10
Case 1 Sputum smear shows a cluster of another type of cell which are large and characterized by rich reticular or vacuolar cytoplasm and large nuclei with coarse chromatin and large nucleoli

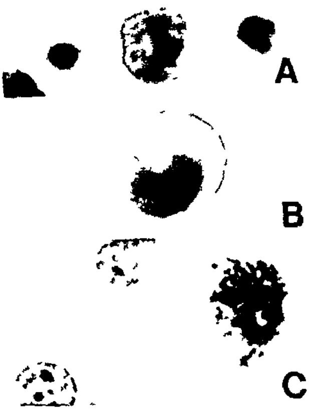


Figure 11
Case 1 (A) A relatively smaller cell with large hyperchromatic nucleus (B) The cytoplasm of a tumor cell is vesiculated (C) Three atypical cells with rich vacuolar cytoplasm one shows abnormal mitosis

tained up to six prominent round or irregularly shaped nucleoli which varied in size

Case 2 Male 62 Years of Age

Six months prior to death the patient was examined at the surgical unit of Fraternity Memorial Hospital because of persistent common-cold like symptoms chest x ray films disclosed a marked enlargement of the right central shadow (Figure 16) Needle biopsy yielded a diagnosis of small-cell anaplastic carcinoma Chemotherapy produced only temporary improvement and the patient eventually developed vena cava superior syndrome and a right pleural effusion

During hospitalization cytologic examination of sputum revealed small-cell carcinoma cells on two occasions and squamous-cell carcinoma cells on two occasions in the terminal stage small-cell carcinoma cells were also demonstrated in the right pleural effusion In retrospect a very vague opacity 2 5 cm ı diameter was detected on the X ray in the left uppe lung field

Gross Pathologic Findings The tumor of the righ lung was a large greyish solid mass measurin 18×13×8 cm situated in the anterior portion of th upper lobe and invading the anterior mediastinun (Figure 17) There was no gross evidence of infiltration into the large bronchial walls suggesting tha the tumor developed primarily in a peripheral bronchus Malignant growth in the right hilar lympl nodes was evident

Another tumor measuring 2 3×2×1 7 cm wa found in the peripheral region of the left lung S_1 (Figure 18) The center of the tumor was necrotic forming a cavity with an uneven inner surface Th

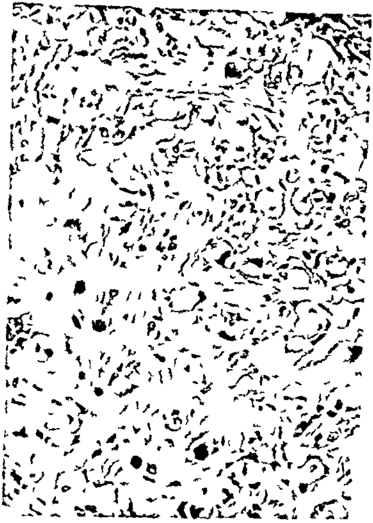


Figure 12
Case 1 second tumor Increased hyaline stroma appear transformed into a cartilage like structure

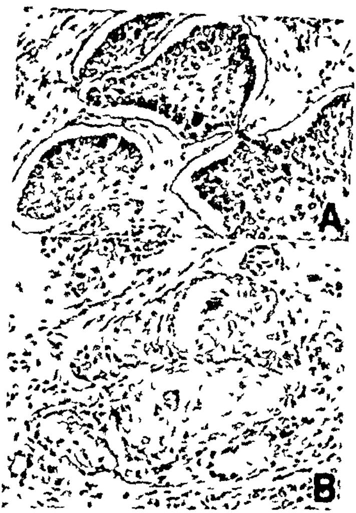


Figure 13
Case 1 second tumor (A) Mucoepidermoid structures are seen in the peripheral area of the second tumor (B) A squamoid pattern is also illustrated within the mucoepidermoid structure

central part of the tumor showed polypoid growth into the bronchial lumen with some infiltration into the bronchial wall There was no gross or microscopic evidence of either regional or remote metastasis

Histologic Findings Histologically the tumor of the right lung revealed morphologic features characteristic of oat-cell carcinoma (Figure 19) The tumor of the left upper lobe was squamous-cell carcinoma (Figure 20) with a prominent tendency to keratinize serial sectioning did not show any undifferentiated lesions within or at the margin of the tumor

Cytologic Findings The small-cell carcinoma cells (Figure 21) found in the cytologic examination of Papanicolaou-stained sputum thoracentesis aspirates and pleural effusion had small irregular spheroid nuclei of 6μm to 11μm (mean 8μm) in the maximum diameter The nuclei contained sporadically distributed coarse granules in finely granular nucleoplasm and nucleoli were not prominent The cytoplasm was noticeably narrow with indistinct cell borders and stained light green

The squamous-cell carcinoma cells (Figure 22) ranged in the maximum diameter from 13μm to 40μm The cytoplasm stained intensely with light green (Figures 22A and 22B) or with orange G (Figure 22C) and had a distinct margin giving the impression of a thick annular ring structure The nuclei of the tumor cells had a coarse margin with coarsely granular or dense nucleoplasm and were situated centrally

In no instance were these two types of tumor cells found in the same specimen

Discussion

Common in the two cases described above was the

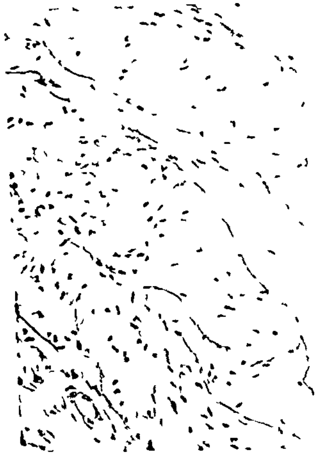


Figure 14
Case 1, second tumor. Myxomatous pattern.

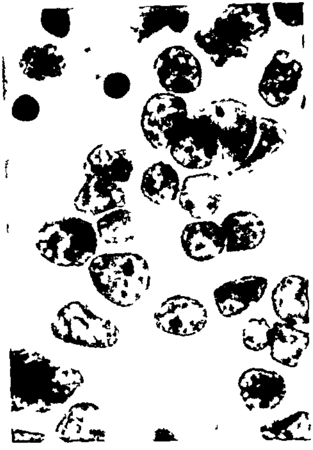


Figure 15
Case 1. Sputum smear shows carcinoma cells of small-cell type.

presence of one extensively spread small-cell anaplastic carcinoma in the lung along with another pulmonary carcinoma which was relatively well circumscribed in the contralateral lung and entirely different from the former histologically. The combinations of small-cell type carcinoma cells with large-cell type or adenocarcinoma type cells as demonstrated in the antemortem cytologic examinations coincided with the postmortem findings.

Incidence of Double Primary Cancers of the Lung

Reports of double primary cancers of the lung date back to 1924, when Beyrenthar first described a case. According to the review of large series of multiple lung cancers [illegible] the incidence was 0.2% to 3.5% of all pulmonary carcinomas. These incidences differ somewhat from the overall incidence of 2.0% to 4.0% for multiple cancers of other organs.[1] However, it seems probable that the incidence of multiple primary cancers of the lung would increase with more detailed examinations, as seen in the reports of Auerbach et al[1] and Suemasu et al.[1] In Japan there have been sporadic reports of double primary cancers of the lung, but no report has dealt with many cases. There have been a total of 44 autopsy cases of lung cancer in the past decade at Fraternity Memorial Hospital, among which only the two cases in this report were double primary cancers of the lung. A total of 117 autopsy cases of lung cancer have been examined at the Department of Pathology, Toho University School of Medicine, during the past ten years, and this series included only one case of double primary cancers of the lung. Thus, double cancers of the lung accounted for 1.9% of the cases of lung cancer examined at these two institutions during the past ten years.

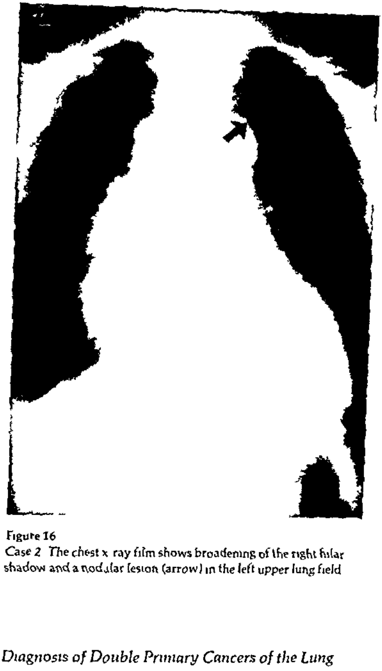


Figure 16
Case 2 The chest x ray film shows broadening of the right hilar shadow and a nodular lesion (arrow) in the left upper lung field

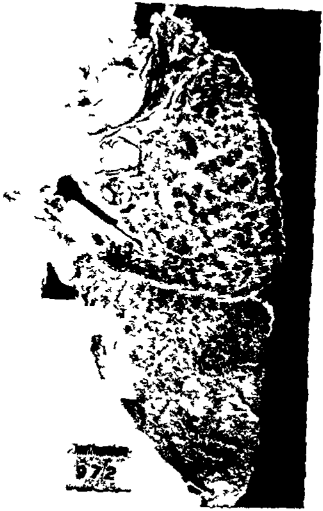


Figure 17
Case 2 The diffusely proliferating tumor in the right lung is situated in the anterior portion of the upper lobe and involves the mediastinum

Diagnosis of Double Primary Cancers of the Lung

According to recent accounts[2,9] Billroth and von *Winiwater in 1883 were the first to propose a definition* of double cancer a slight modification of which was suggested in 1932 by Warren and Gates In any case it is essential that the second tumor never be a metastasis of the first tumor In attempting a diagnosis of a double cancer therefore differences in histologic types between the two tumors would constitute one of the most substantial grounds[10] The pathologist should be cautious however in making a histologic diagnosis of double cancers of the lung inas much as lung cancers are characterized by diverse histologic features One must consider the manner of metastasis[10] and clinical parameters such as the disparity in doubling time of tumors in cases where similar histologic types are encountered

Because squamous metaplasia and gland like structures are occasionally found even in small-cell anaplastic carcinoma the possibility exists that even when the second tumor is a squamous-cell carcinoma or an adenocarcinoma it may merely represent an accentuation of a particular aspect of the first tumor In his histologic study of material from 100 surgically resected lung cancers Shimosato[12] reported that a single histologic type was noted in nearly 60% of the cases studied and that in the majority of tumors showing histologic pleomorphism combinations of two of the four basic types were evident squamous cell carcinoma adenocarcinoma small-cell carcinoma and large-cell carcinoma The combinations were characterized primarily by the inclusion of large-cell anaplastic carcinoma He stated further that transitional forms were usually noted between the respective histologic types in such cases Thus when malig

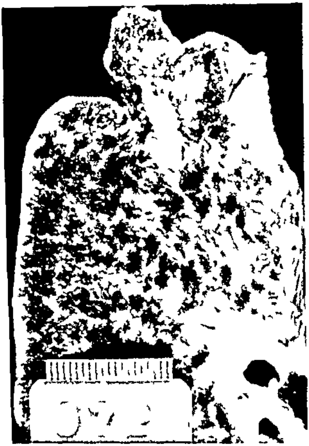


Figure 18
Case [illegible]. An abnormal nodular shadow on the left upper lung field in Figure 16 correspond to a peripheral-type tumor related to B

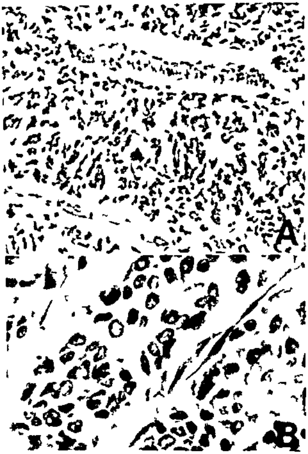
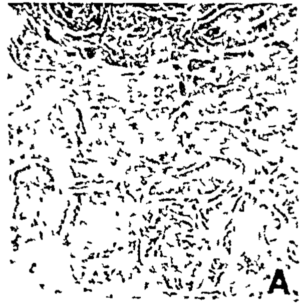


Figure 19
Case 2. (A) The right lung tumor is a small-cell anaplastic carcinoma, oat-cell type



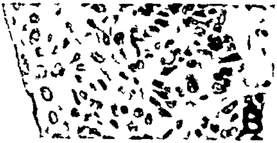


Figure 20
Case [illegible]. (A) The tumor in the left upper lobe shows moderately differentiated squamous cell carcinoma. The upper half of the photograph reveals keratotic and necrotic lesions. (B) The mucosa of B is also involved by carcinoma.

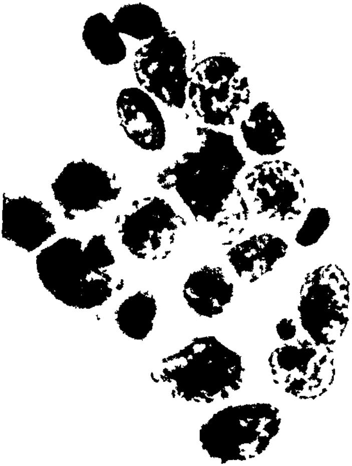


Figure 21
Case 2 Aspiration smear from the mediastinum shows a sheet of carcinoma cells of the small-cell type

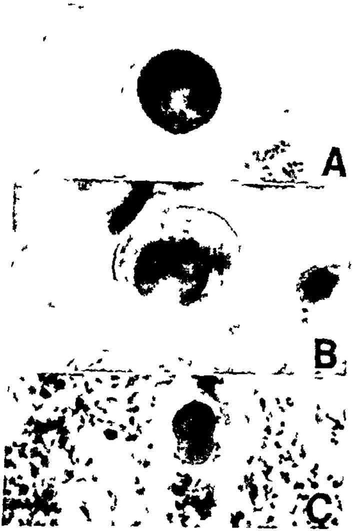


Figure 22
Case 2 Cells seen in the smear materials of sputum (A and B) Cells characterized by laminated cytoplasm (C) A dyskeratotic cell

nant cells of two or more types are found cytologically and in addition a transitional interrelationship cannot be found between them it is important to postulate that there may exist a plurality of primary carcinomas corresponding histologically to the respective types of malignant cells

Malignant Mixed Tumors of the Lung

In the WHO classification of lung tumors[8] mixed tumors are classified in the category mixed tumor and carcinosarcomas

The mixed tumor of the salivary gland type is classified in the same category The two cases reported by *Dayne et al*[4] and the one described by Davis[3] fall *in this* category exactly but histologically they presented morphologic characteristics that should be regarded as benign In Japan one case[1] of malignant mixed tumor of the salivary-gland type has been illustrated in the *Atlas of Lung Cancer* and two cases[5 7] in the *Atlas of Exfoliative Cytology of Rare Cases* one case has been reported in detail by Ushijima [15]

The second tumor in the first case in this report seems to fall into this category although the question of whether it was initially a benign growth which changed afterwards into a malignant form or whether it grew malignant from the beginning is unknown While the mixed tumors in the four cases previously reported in this country and the one described above have no benign aspect all other cases reported in the literature were of a benign form This indicates that there may exist both a benign and a malignant form of mixed tumor of the salivary-gland type

Cytologic Determination of the Histologic Typing of Lung Cancers

The discovery of tumor cells of two or more different types in the sputum may not seem unusual when one thinks of the characteristic diversity of microscopic features of lung cancers From the observations of Shimosato[12] however the appearance of tumor cells of different types in either the sputum or other specimens of the lung could be interpreted as representing the possible presence of a plurality of tumors corresponding to the respective malignant cell types found in the cytologic examination[*] and thus the importance of searching for a second tumor should be stressed Attention should also be directed to the significance of repeated cytologic examination of the sputum from the viewpoint of detecting double primary lung cancers Constant survey of specimens obtained from a patient in routine screening may lead to the discovery of such cases

However it would be extremely difficult if not impossible to diagnose such intricate histologic features as in the second tumor in case 1 of this report in which cells found in the sputum corresponding to the malignant mixed tumor were diagnosed cytologically as large-cell type or adenocarcinoma type This may be inevitable insofar as the materials with which clinical cytology deals hardly permit a fully satisfactory structural differentiation

References

1 Auerbach O Stout AP Hammond EC Garfinkel L Multiple primary bronchial carcinomas Cancer 20 699 705 1967

2 Baba K Shimosato Y Watanabe S Tajima T Statistics or multiple primary cancer and related problems Jpn J Cancer Clin 17 4 4-436 1971 (in Japanese)

3 Davis PW Briggs JC Seal RME Storring FK Benign and malignant mixed tumors of the lung Thorax 7 657-673 1972

4 Payne WS Schier J Woolner LB Mixed tumors of the bronchus (salivary gland type) J Thorac Cardiovasc Surg 49 663 668 1965

5 Fukushima N Malignant mixed tumor (salivary gland type o the bronchus) *In* Atlas of Exfoliative Cytology of Rare Cases Edited by Y Tenjin M Hattori Tokyo Bunkodo 1972 pp 76-77 (in Japanese)

6 Grunze H A critical review and evaluation of cytodiagnosis in chest diseases Acta Cytol 4 175 198 1960

7 Koketsu H Malignant mixed tumor of the lung (salivary gland type) *In* Atlas of Exfoliative Cytology of Rare Cases Edited by Y Tenjin M Hattori Tokyo Bunkodo 1972 pp 78 79 (in Japanese)

8 Kreyberg L Mixed tumor and carcinosarcomas *In* Histological Typing of Lung Tumors Geneva World Health Organization 1967 pp 4 5

9 Langston HT Sherrick JC Bilateral simultaneous bronchogenic carcinoma J Thorac Cardiovasc Surg 43 74 751 1962

10 Martini N Melamed MR Multiple primary lung cancers J Thorac Cardiovasc Surg 70 606-612 1975

11 Rohwedder JJ Weatherbee L Multiple primary bronchogenic carcinoma with a review of the literature Am Rev Respir D 109 435-445 1974

12 Shimosato Y Pathology of lung cancer *In* Atlas of Lung Cancers Edited by M Kuru S Ishikawa Tokyo Nakayama shoten 1968 pp 99 313 (in Japanese)

13 S emau K Shimosato Y Ishikawa S Multiple minute cancers of major bronchi A case report J Thorac Cardiovasc Surg 68 664-677 1974

14 Thoma GW The incidence and significance of multiple primary malignant tumors Am J Med Sci 47 427-430 1964

15 Uchijima H Fujiwara K Yamaguchi T Hatani K Kakihara R Suenaga Y A case of lung cancer of malignant mixed tumor origin Jpn J Cancer Clin 21 13 7 1333 1975 (in Japanese)

Needle Biopsy Diagnosis of Solitary Amyloid Nodule of the Lung

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Carlos Abramowsky, M D
Alan M Cohen, M D
Geraldine Horak, C T (ASCP) C T (IAC)

Solitary amyloid nodule of the lung is an unusual form of localized amyloidosis which is rarely diagnosed prior to surgical resection This report describes a case of solitary amyloid nodule of the lung which was suggested prospectively on the initial needle biopsy The histologic and cytologic features of the lesion appear to be characteristic and may allow a definitive diagnosis to be made on needle biopsy The differential diagnosis from necrotic tumor and medullary carcinoma is described

The subsequently resected nodule was studied by both electron microscopy and immunofluorescent techniques Immunofluorescent staining failed to confirm an origin of the lesion from a single clone of plasma cells

Needle biopsy and fine needle aspiration have been utilized to detect amyloid in the kidney synovial fluid[5] spleen[15] skin[17] and bone marrow[4] in generalized amyloidosis and the thyroid in medullary carcinoma[10] and the lung in nodular amyloidosis[3,16] In these cases the diagnosis was made by Congo red staining with positive green birefringence of the aspirated and biopsied material There is no reference in these cases, nor in standard textbooks of cytology[4,11] to the diagnosis of amyloid in routine cytologic preparations

Solitary amyloid nodule of the lung is a rare form of localized amyloidosis In the cases of solitary[16] and multiple[3] amyloid nodules of the lung in which needle biopsy had been attempted the diagnosis was made only retrospectively following excision of the lesion

This paper reports a case of solitary pulmonary amyloid nodule in which the diagnosis was suggested on the initial needle biopsy which was studied both histologically and cytologically Histologic immunofluorescent and electron microscopic studies were performed subsequently on the resected specimen

Case Report

The patient was a 64 year-old woman with a several day history of nausea and vomiting She had suffered from nondeforming arthritis for ten years it initially had been believed to be seronegative rheumatoid arthritis Other symptoms included skin rash upon sun exposure Raynaud s phenomenon and iritis In the past she had been treated with a variety of medications including methotrexate nitrogen mustard and nonsteroidal antiinflammatory agents she was taking prednisone 30 mg q d at the time of admission Recent laboratory studies included a positive antinuclear antibody test (1 1 000) positive anti DNA antibody titers (1 40) and a positive LE cell prep On serum immunoelectrophoresis there was a polyclonal increase in IgG and IgA On the basis of these studies the clinical diagnosis was changed to systemic lupus erythematosus There was no evidence of multiple myeloma or Bence Jones proteinuria Renal function tests were normal

On physical exam the patient was lethargic and ill appearing with a temperature of 37 6 C blood pressure of 140/80 mm Hg pulse of 100 per minute and

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respiratory rate of 18 per minute There was a grade II/IV systolic ejection murmur The patient had minimal hand deformities

After admission the patient developed a right pleural effusion On thoracentesis 200 cc of clear yellow sterile fluid was removed After drainage of the effusion a noncalcified poorly delimited 2-cm coin lesion was noted radiographically in the right lower lobe By tomography the nodule was situated within the lung parenchyma Needle biopsy was performed with an 18-gauge Lee biopsy needle Tissue was obtained for routine histology and saline washings of the nodule and rinsings of the needle and syringe were also submitted for cytologic examination

The patient subsequently underwent thoracotomy and resection of the nodule No other parenchymal or pleural disease was seen at the time of surgery The patient has had no recurrence of the lesion over a three month follow up period

Results

Needle Biopsy

In the needle biopsy stained with hematoxylin and eosin were fragments of eosinophilic oligocellular amorphous material in a background of red cells and basophilic granular debris Mature bone spicules were present some of which were intimately associated with the eosinophilic fragments (Figure 1A) The fragments were stained metachromatically with crystal violet and gave a green birefringence under polarized light with Congo red staining The predominant cellular elements were mononuclear cells with pyknotic nuclei Lymphocytes macrophages plasma cells and occasional polymorphonuclear leukocytes were also present

Cytology

The cytocentrifuged cytology specimen stained with EA65 contained numerous waxy green to dark blue acellular fragments in a bloody background (Figure 1B) The fragments measured from 6 5μm to 500μm with most in the range of 70μm to 80μm They varied in shape from sharply angulated to scalloped Some had central eosinophilia and prominent fissures (Figure 1C) Following excision of the nodule the cytology smear was decolorized and restained with Congo red and crystal violet both of which were positive for amyloid

Resected Specimen

The resected specimen was an ill-defined yellow

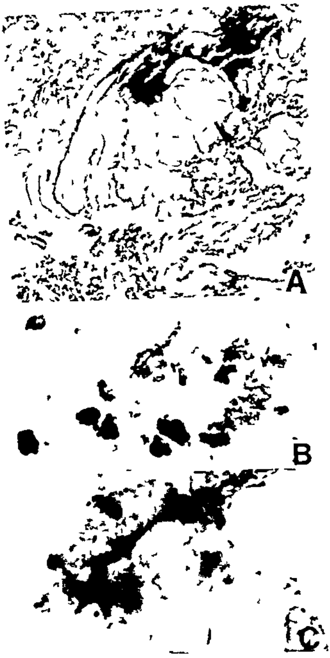

Figure 1
(A) Needle biopsy of solitary amyloid nodule To the right of center is a calcified bony spicule surrounded by multiple irregular fragments of amyloid (arrow) A slender fragment of dense collagenous connective tissue is seen in the right lower corner The background is granular and hemorrhagic (hematoxylin and eosin original magnification × 160) (B) Cytologic smear of washings from solitary nodule There are multiple irregular dense acellular fragments with a hemorrhagic sparsely cellular background (EA65 original magnification × 100) (C) Detailed view of an amyloid fragment seen in the cytologic smear It has a wavy scalloped configuration with a prominent central fissure (EA65 original magnification × 350)

brown, firm, gritty, subpleural nodule measuring 1.5 cm in diameter. The surrounding lung parenchyma was grossly unremarkable. Microscopically, the poorly circumscribed mass was composed of confluent, amorphous, eosinophilic nodules separated by focally ossified fibrous septa with dense clusters of lymphocytes and plasma cells and occasional multinucleated giant cells (Figure 2). The eosinophilic material extended into adjacent alveolar septa and surrounding blood vessels. Congo red and crystal violet stains were positive for amyloid.

On electron microscopy the acellular material consisted of an irregularly woven fibrillar meshwork. The fibrils had a finely beaded appearance and measured approximately 80 Å in diameter.

Immunofluorescent staining with anti-IgG, IgA, IgM, C 3 and gamma globulin demonstrated bright green fluorescence of the amyloid and cytoplasmic staining of plasma cells with IgG. Type-specific staining with antisera for lambda and kappa light chains demonstrated both types within the amyloid and plasma cells.

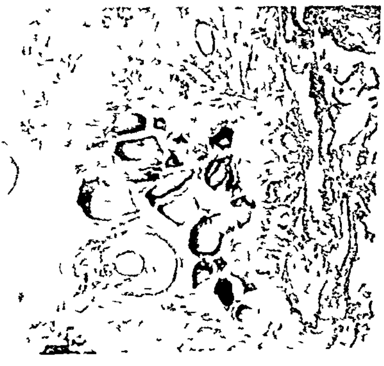

Figure 2
Solitary amyloid nodule of the lung. The left half of the field is occupied by a poorly delimited nodule consisting of amyloid, fibrous connective tissue, bone spicules, lymphocytes and plasma cells. The amyloid extends into the adjacent alveolar septa and is associated with a chronic interstitial pneumonitis (hematoxylin and eosin, original magnification × 100).

Discussion

Clinically, patients with solitary amyloid nodule of the lung are usually over 50 years old and without associated illnesses.[1] There is no sex predilection. Patients are either asymptomatic or have nonspecific respiratory complaints. Often the lesion is discovered incidentally on routine chest radiographs; it is usually subpleural and may occur in any lobe. There is no association with concomitant or subsequent multiple myeloma or generalized amyloidosis. In the two cases with prolonged follow-up, one patient was followed six years without recurrence[7] while in the other the lesion recurred after nine years.[4] The etiology is unknown, but it may be the result of immunoglobulin secretion by a focal abnormal clone of plasma cells.[14]

In all previously reported cases the diagnosis has been made following resection of the nodule, which has a characteristic pattern similar to this case. The previously reported experience with needle biopsy is limited to two cases: in one, the biopsy of a solitary amyloid nodule was initially interpreted as necrotic material[16]; in the other, needle biopsy was initially interpreted as atypical epithelial cells suggestive of squamous cell carcinoma.[3] Only after open lung resection was the needle biopsy in the latter case restained with Congo red, demonstrating the amyloid composition of the eosinophilic fragments. Bone spicules were not present in either of these needle biopsies although bone was present in four of the reported cases of solitary amyloid nodule.

In this case the amorphous material in the EA65-stained cytology smears was morphologically similar to the amyloid fragments in the needle biopsy. In both preparations the acellular material had a waxy, scalloped appearance with rough angulations and occasional fissures. Destaining the cytology smear and restaining with Congo red showed green birefringence under polarized light, further substantiating that the material was amyloid.

The amyloid stained with EA65 could be distinguished from certain other acellular material which might be observed in a fine needle pulmonary aspirate. Pulmonary corpora amylacae, which may stain positively with Congo red, are generally pale red to bluish red and have a uniform, round to oval, waxy appearance.[13] Psammoma bodies are red, have a laminated, scalloped appearance and are usually associated with cytologically malignant cells.[13] Bronchial mucus may have the same tinctorial properties as amyloid but is usually less dense and more stringy and may be more abundant.

Although the appearance of amyloid in Papanicolaou-stained preparations is not described in the standard references of cytology an amorphous material similar in morphology and tinctorial properties to the material in this case is sometimes noted in joint aspirates from patients with rheumatoid arthritis [11] Bluish amorphous masses are also seen occasionally in cervical scrapings from normal women The cytologic features of the amorphous material in this case cannot therefore be considered specific for amyloid In the proper clinical setting however this cytologic appearance can be highly suggestive and prompt further studies

The electron microscopic studies in this case were diagnostic of amyloid and resembled the findings in one other case of nodular pulmonary amyloidosis [12] The immunofluorescent studies failed to confirm the speculations that the solitary amyloid nodule is derived from light chains secreted by a monoclonal proliferation of plasma cells The positive immunofluorescence of the amyloid with anti IgG does not moreover imply an immunologic origin This type of staining may represent absorption of immunoglobulins to the amyloid meshwork [2]

On the basis of this experience it seems that amyloid nodule of the lung may be diagnosed by needle biopsy more frequently in the future Recognition of this fact may permit some patients to be spared thoracotomy However amyloid can be a component of primary lung neoplasms [6] Another possibility in the differential diagnosis is metastatic origin from medullary carcinoma of the thyroid or metastatic islet cell tumor although these are often functioning neoplasms and usually have detectable primaries by the time they have metastasized to the lung Although these lesions should contain neoplastic cells it is theoretically possible that they might be missed due to sampling error In those patients who can tolerate thoracotomy therefore it might be best to rule out malignancy by resecting the entire lesion Experience with needle biopsy of such neoplastic lesions is necessary before a claim can be made that solitary amyloid nodule of the lung can be diagnosed unequivocally by needle biopsy

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References

1 Brauner GJ Bazzaz FA Mihm MC Acquired bullous disease of the skin and solitary amyloidoma of the lung Am J Med 57 978-986 1974

2 Cathcart ES Cohen AS The relation between isolated human amyloid fibrils and human gamma globulin and its subunits J Immunol 96 239 252 1966

3 Dahlgren SE Lewenhaupt A Ovenfors L Fine needle biopsy diagnosis in nodular pulmonary amyloidosis Acta Pathol Microbiol Scand [A] 78 1 5 1970

4 Dyke PC, Demaray MJ Delavan JW Rasmussen RA Pulmonary amyloidoma Am J Clin Pathol 43 301-305 1974

5 Gordon DA Pruzanski W Ogryzlo MA Synovial fluid examination for the diagnosis of amyloidosis Ann Rheum Dis 32 428-430 1973

6 Gordon HW Miller R Mittman C Medullary carcinoma of the lung with amyloid stroma A counterpart of medullary carcinoma of the thyroid Human Pathol 4 431-436 1973

7 Hayes WT Bernhardt H Solitary amyloid mass of the lung Report of a case with 6 year follow-up Cancer 24 820-825 1969

8 Koss LG Diagnostic Cytology and Its Histopathologic Bases Second edition Philadelphia JB Lippincott Co 1968

9 Kyle RA Pease GL Richmond H Sullivan L Bone marrow aspiration in the antemortem diagnosis of primary systemic amyloidosis Am J Clin Pathol 45 252 257 1965

10 Ljungberg O Cytologic diagnosis of medullary carcinoma of the thyroid gland with special regard to the demonstration of amyloid in smears of fine needle aspirates Acta Cytol 16 253 255 1972

11 McGrew EA Nanos S The cytology of serous effusions *In* A Manual of Cytotechnology Edited by CM Keebler JW Reagan Fourth edition Chicago American Society of Clinical Pathology 1975 p 199

12 Makinen J Nickels J Haluunen PEA Amyloid tumor of the lung Acta Pathol Microbiol Scand [A] 85 907-911 1977

13 Naib ZN Exfoliative Cytopathology Second edition Boston Little Brown & Co Inc 1976 pp 248 307 308

14 Page DL Isersky C Harada M Glenner G Immunoglobulin origin of localized nodular pulmonary amyloidosis Resp Exp Med 159 75 1977

15 Pasternak A Fine needle aspiration biopsy of spleen in the diagnosis of generalized amyloidosis Br Med J [illegible] 0-2[illegible] 1974

16 Skageth E Jr Normann T Localized amyloid tumor of the lung Scand J Thorac Cardiovasc Surg 4 135-138 1970

17 Westermark P Stenkvist B Diagnosis of secondary generalized amyloidosis by fine needle biopsy of the skin Acta Med Scand 190 453-454 1971

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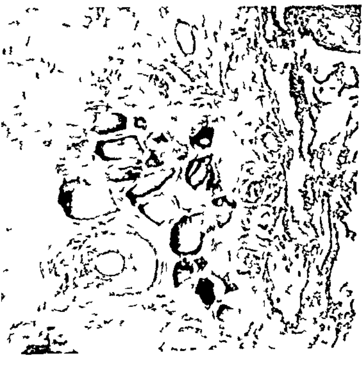

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In this case the amorphous material in the EA65-stained cytology smears was morphologically similar to the amyloid fragments in the needle biopsy In both preparations the acellular material had a waxy scalloped appearance with rough angulations and occasional fissures Destaining the cytology smear and restaining with Congo red showed green birefringence under polarized light further substantiating that the material was amyloid

The amyloid stained with EA65 could be distinguished from certain other acellular material which might be observed in a fine needle pulmonary aspirate Pulmonary corpora amylacae which may stain positively with Congo red are generally pale red to bluish red and have a uniform round to oval waxy appearance [13] Psammoma bodies are red have a laminated scalloped appearance and are usually associated with cytologically malignant cells [13] Bronchial mucus may have the same tinctorial properties as amyloid but is usually less dense and more stringy and may be more abundant

Although the appearance of amyloid in Papanicolaou-stained preparations is not described in the standard references of cytology an amorphous material similar in morphology and tinctorial properties to the material in this case is sometimes noted in joint aspirates from patients with rheumatoid arthritis [11] Bluish amorphous masses are also seen occasionally in cervical scrapings from normal women The cytologic features of the amorphous material in this case cannot therefore be considered specific for amyloid In the proper clinical setting however this cytologic appearance can be highly suggestive and prompt further studies

The electron microscopic studies in this case were diagnostic of amyloid and resembled the findings in one other case of nodular pulmonary amyloidosis [12] The immunofluorescent studies failed to confirm the speculations that the solitary amyloid nodule is derived from light chains secreted by a monoclonal proliferation of plasma cells The positive immunofluorescence of the amyloid with anti IgG does not moreover imply an immunologic origin This type of staining may represent absorption of immunoglobulins to the amyloid meshwork [2]

On the basis of this experience it seems that amyloid nodule of the lung may be diagnosed by needle biopsy more frequently in the future Recognition of this fact may permit some patients to be spared thoracotomy However amyloid can be a component of primary lung neoplasms Another possibility in the differential diagnosis is metastatic origin from medullary carcinoma of the thyroid or metastatic islet cell tumor although these are often functioning neoplasms and usually have detectable primaries by the time they have metastasized to the lung Although these lesions should contain neoplastic cells it is theoretically possible that they might be missed due to sampling error In those patients who can tolerate thoracotomy therefore it might be best to rule out malignancy by resecting the entire lesion Experience with needle biopsy of such neoplastic lesions is necessary before a claim can be made that solitary amyloid nodule of the lung can be diagnosed unequivocally by needle biopsy

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References

1 Brauner GJ Bazzaz FA Mihm MC Acquired bullous disease of the skin and solitary amyloidoma of the lung Am J Med 57 978-986 1974

2 Cathcart ES Cohen AS The relation between isolated human amyloid fibrils and human gamma globulin and its subunits J Immunol 96 239 252 1966

3 Dahlgren SE Lewenhaupt A Ovenfors L Fine needle biopsy diagnosis in nodular pulmonary amyloidosis Acta Pathol Microbiol Scand [A] 78 1 5 1970

4 Dyke PC Demaray MJ Delavan JW Rasmussen RA Pulmonary amyloidoma Am J Clin Pathol 43 301 305 1974

5 Gordon DA Pruzanski W Ogryzlo MA Synovial fluid examination for the diagnosis of amyloidosis Ann Rheum Dis 32 428-430 1973

6 Gordon HW Miller R Mittman C Medullary carcinoma of the lung with amyloid stroma A counterpart of medullary carcinoma of the thyroid Human Pathol 4 431-436 1973

7 Hayes WT Bernhardt H Solitary amyloid mass of the lung Report of a case with 6 year follow-up Cancer 24 8 0-825 1969

8 Koss LG Diagnostic Cytology and Its Histopathologic Bases Second edition Philadelphia JB Lippincott Co 1968

9 Kyle RA Pease GL Richmond H Sullivan L Bone marrow aspiration in the antemortem diagnosis of primary systemic amyloidosis Am J Clin Pathol 43 252 257 1965

10 Ljungberg O Cytologic diagnosis of medullary carcinoma of the thyroid gland with special regard to the demonstration of amyloid in smears of fine needle aspirates Acta Cytol 16 253 255 1972

11 McGrew EA Nanos S The cytology of serous effusions In A Manual of Cytotechnology Edited by CM Keebler JW Reagan Fourth edition Chicago American Society of Clinical Pathology 1975 p 199

12 Makinen J Nickels J Haluunen PEA Amyloid tumor of the lung Acta Pathol Microbiol Scand [A] 85 907-911 1977

13 Naib ZN Exfoliative Cytopathology Second edition Boston Little Brown & Co Inc 1976 pp 248 307 308

14 Page DL, Isersky C Harada M Glenner G Immunoglobulin origin of localized nodular pulmonary amyloidosis Resp Exp Med 159 75 1972

15 Pasternak A Fine needle aspiration biopsy of spleen in the diagnosis of generalized amyloidosis Br Med J 2 20-22 1974

16 Skagseth E Jr Normann T Localized amyloid tumor of the lung Scand J Thorac Cardiovasc Surg 4 135-138 1970

17 Westermark P Stenkvist B Diagnosis of secondary generalized amyloidosis by fine needle biopsy of the skin Acta Med Scand 190 453-454 1971

Malignant Histiocytosis Showing Ascites and Recurrent Meningeal Infiltration

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Report is made of a case of malignant histiocytosis (MH) in which abdominal effusion and recurrent meningeal symptoms due to tumor cell infiltration appeared The diagnosis of malignant histiocytosis was made by histologic examination of biopsy materials from the right submandibular mass The specimens from the ascites and the liquor from a lumbar puncture showed three types of cells normal-appearing histiocytes large atypical histiocytic cells and bi and multinucleated cells Phagocytosis was observed in the normal-appearing histiocytes and the large atypical cells similar findings were observed in the specimens from marrow The autopsy findings showed a systemic diffuse nontumorous proliferation of morphologically atypical histiocytes Although ascites or meningeal symptoms caused by infiltration of neoplastic histiocytes are extremely rare the possibility of MH should be considered in diagnosing conditions such as observed in this case

Malignant histiocytosis (MH) is a neoplastic proliferation of morphologically atypical histiocytes [12] In 1939 Scott and Robb-Smith[14] introduced the term 'histiocytic medullary reticulosis (HMR) and defined the condition as a progressive cellular hyperplasia of the hemopoietic and lymphatic tissue Later Marshall claimed that HMR was a neoplastic disease [10] In 1966 Rappaport introduced the term 'MH for the condition characterized by a systemic progressive invasive proliferation of morphologically atypical histiocytes [12] We present here a case of MH *with ascites and meningeal involvement*

Case Report

A 14 year-old girl in good health until the present illness suffered from cough and fever beginning 24 January 1978 and was hospitalized on 8 February 1978 On admission superficial lymphadenopathy was not noticed except for the right submandibular mass which was about the size of a thumb There was no hepatosplenomegaly Laboratory data showed slightly hypochromic anemia and a WBC of 4 600/cu mm with a differential of 12% bands 54% neutrophils 1% eosinophils 6% monocytes and 27% lymphocytes A bone marrow aspirate showed hypocellularity but no atypical cells Platelets were not decreased Repeated bone marrow punctures revealed a few atypical histiocytic cells Although several antibiotics were administered abdominal distention began to develop Abdominal puncture yielded an effusion in which atypical cells were intermingled with normal appearing histiocytes Biopsy findings from the submandibular mass revealed a diffuse nontumorous proliferation of atypical histiocytic cells The histologic diagnosis of MH was made on 3 March 1978 and chemotherapy (VEMP) was begun the next day

The patient s temperature returned to normal and

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the ascites disappeared completely by four days after the onset of VEMP therapy On 1 April 1978 the patient began to complain of headaches A turbid liquor obtained by lumbar puncture contained many atypical histiocytic cells The meningeal infiltration by atypical histiocytic cells recurred three times during her course and each time was cleared by several intrameningeal injections of methotrexate (10 to 20 mg) The patient died of massive gastrointestinal bleeding due to hemorrhagic diathesis on 22 October 1978

Cytologic Findings

Except for the high content of lymphocytes in the ascites cytologic findings of proliferating cells in the ascites and liquor were almost identical The proliferating cells did not form cohesive masses Three types of cells were observed normal appearing histiocytes large atypical cells with various degrees of light-green staining in the cytoplasm and bi or multinucleated cells In the relatively abundant irregularly shaped cytoplasm small vacuoles were frequently observed The large atypical cells had round or irregularly shaped nuclei varying in size with clear nuclear membranes and one or more large irregularly shaped nucleoli The nuclear chromatin was increased in amount distributed unevenly and showed reticular or small vesicular patterns (Figure 1) Bi or multinucleated cells with strong light-green staining in the cytoplasm resembled Reed Sternberg cells Mitotic figures were observed frequently Both the normal appearing histiocytes and the large atypical cells showed phagocytosis (Figure 2) In Wright Giemsa-stained marrow films the large atypical cells showed active phagocytosis (Figure 3)

Biopsy Findings

A piece of the right submandibular mass consisting of part of a lymph node with the surrounding connective tissue was obtained by a surgical procedure The peripheral sinus was partially preserved but the lymphatic stroma was permeated by neoplastic appearing large histiocytic cells The capsular and pericapsular connective tissue had been largely replaced by fibrous tissue In the lymphatic stroma tumor cells were mixed with lymphocytes and plasma cells and the figure was not monomorphic (Figure 4) The same three types of proliferating cells identified by cytology were observed Many cells possessed vacuoles in their cytoplasm (Figure 5) The atypical histiocytes showed a lack of cohesiveness Phagocytic and mitotic figures were seldom seen

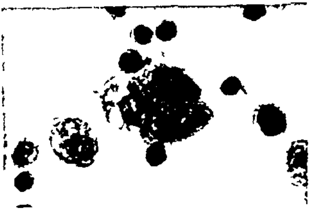

Figure 1
Ascites Multinucleated giant cell large atypical cells and normal appearing histiocytes (Papanicolaou stain × 1 000)

Figure 2
A large atypical cell shows phagocytosis (Papanicolaou stain × 1 000)

Figure 3
Sternal marrow puncture Two large histiocytic cells show active phagocytosis (Wright-Giemsa stain × 1 000)

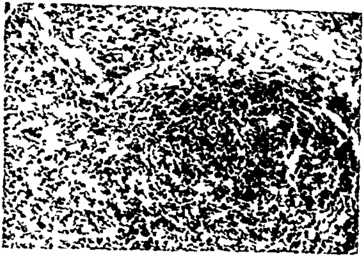

Figure 4
Low power view of lymph node showing infiltration of tumor cells in the lymphoid stroma and capsule Sinus is preserved Lymphocytes and plasma cells are mixed with tumor cells and the figure is not monomorphic (hematoxylin and eosin × 40)

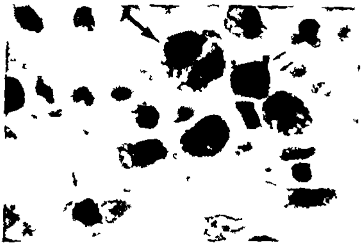

Figure 5
High power view of lymphoid stroma composed of normal appearing histiocytes (small arrow) large atypical cells (double arrow) and binucleated cells (large arrow) Intracytoplasmic vacuoles are observed (hematoxylin and eosin × 1 000)

Autopsy Findings

Gross examination showed hemorrhagic diathesis as a prominent feature but jaundice was not observed There was a hard slightly elevated area 5×6 cm in size in the right inguinal region The degree of lymphadenopathy was slight The liver and spleen were enlarged weighing 2 660 and 380 gm respectively Gross tumor nodule formation was not observed Bone marrows showed diminution of red color The central nervous system was not examined

Microscopically atypical histiocytic cells were found infiltrating the lymph nodes liver spleen jejunum thymus and bone marrows In the lymph nodes normal architecture was obscured by massive fibrosis in which a few histiocytic cells were observed In the spleen histiocytic cells proliferated primarily in the red pulp this cell proliferation partially or completely obliterated the white pulp The three types of proliferating cells were observed as in the biopsy findings and erythrophagocytosis was observed In the liver histiocytic cells had infiltrated mainly the portal region but the infiltration was minimal The greater part of the hepatomegaly was caused by *toxic injury due to chemotherapeutic* agents Bone marrows were hypocellular with diffuse proliferation of histiocytic cells Erythrophagocytosis was a prominent feature in the bone marrows

Discussion

MH is a neoplastic systemic proliferation of morphologically atypical histiocytes[12] The same condition has been reported under a variety of terms including aleukemic reticulosis histiocytic reticulosis malignant leukemic reticulohistiocytosis malignant reticulosis and histiocytic leukemia Many reports appear in the world literature under the terms *HMR* and *MH*[1,4,11,13,19] The relationship of MH and HMR has been disputed but it is now generally agreed that *MH and HMR should be regarded as* synonymous[2,7] *We did not locate a previously reported case of MH with neoplastic-appearing histio*cytic cells in ascites or liquor

The diagnosis of MH is usually based on histologic findings of biopsy or in many cases autopsy materials The diagnostic value of marrow examination has been reported[3]

The presence of the three types of proliferating cells described above and their phagocytic activity are characteristic features in the cytologic diagnosis of MH MH can be distinguished cytologically from malignant lymphoma (histiocytic type) by the rarity of these features in the latter[12,17]

Although as described above ascites or meningeal symptoms caused by infiltration of neoplastic histiocytes may be extremely rare MH should be considered in dealing with a condition with a fever of unknown etiology no evident tumorous lesion and laboratory data similar to those reported here

References

1 Abele DS Griffin TB Histiocytic medullary reticulosis Arch Dermatol 106 319 329 1972

2 Byrne GE Rappaport H Malignant histiocytosis *In* Malignant Diseases of the Hematopoietic System Gann Monograph on Cancer Research 15 Tokyo University of Tokyo Press 1973 pp 145 162

3 Chih Fei Y Chung Hong TH Huai Teh H Teh Tsung T Hsi hsien K Chin Tong Y Chieh L Histiocytic medullary reticulosis Chin Med J 80 466-474 1960

4 C v n H Okazaki K Histiocytic medullary reticulosis Report of two cases Arch Intern Med 94 375 383 1954

5 Friedman RM Steigbigel NH Histiocytic medullary reticulosis Am J Med 38 130-133 1965

6 Greenberg E Cohen DM Pease GL Kyle RA Histiocytic medullary reticulosis Mayo Clin Proc 37 271 283 196

7 Huhn D Meister P Malignant histiocytosis Morphologic and cytochemical findings Cancer 4 1341 1349 1978

8 Kageyama K Noguchi H Histiocytic medullary reticulosis Acta Pathol Jpn 14 31 237 1964

9 McGovern VJ Morrow AW Thomson EF A case of histiocytic medullary reticulosis J Pathol Bacteriol 63 340-343 1951

10 Marshall AHE Histiocytic medullary reticulosis J Pathol Bacteriol 71 61 71 1956

11 Persaud V Wood JK Histiocytic medullary reticulosis Report of the first case in Jamaica Am J Clin Pathol 48 396-400 1967

12 Rappaport H Tumors of the Hematopoietic System *In* Atlas of Tumor Pathology Edited by LG Koss Washington DC Armed Forces Institute of Pathology 1966 pp 48-63 99 101

13 Rilke F Carbone C Mussumeci R Pilotti S De Lena M Bonadonna G Malignant histiocytosis A clinicopathologic study of 18 consecutive cases Tumori 64 11 227 1978

14 Scott RB Robb Smith AHT Histiocytic medullary reticulosis Lancet 2 194 198 1939

15 Serck Hanssen A Purch t GP Histiocytic medullary reticulosis A report of 14 cases from Uganda Br J Cancer 22 506-516 1968

16 Vaithianathan V Fishkin S Gruhn JG Histiocytic medullary reticulosis Am J Clin Pathol 47 160-166 1967

17 Warnke RA Kim H Dorfman RF Malignant histiocytosis (histiocytic medullary reticulosis) I Clinicopathologic study of 9 cases Cancer 35 15 230 1975

18 Zak FG Rubin E Histiocytic medullary reticulosis Am J Med 31 813-819 1961

19 Zawadzki ZA Pena CE Fishkin ER Histiocytic medullary reticulosis Case report with electron microscopic study Acta Hematol 42 50-57 1969

Cytogenetic Analysis of an Immunogenic Mutant of the L5178Y Lymphoma

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A mutant of the uniformly lethal L5178Y lymphoma called the L5178Y/Manitoba (L5178Y/M) was rejected after subcutaneous challenge in syngeneic DBA/2 mice Karyotypic analysis revealed that the parent L5178Y lymphoma had four chromosome markers with the mutant L5178Y/M sharing one of them as well as possessing two distinguishing markers One diploid and two hypotetraploid clones were isolated from the L5178Y/M they contained all the marker chromosomes and were also rejected by the syngeneic host In addition to the shared chromosome markers the L5178Y/M possessed antigens in common with the parent L5178Y DBA/2 mice made immune to the mutant by subcutaneous immunization were able to slow the growth of the parent tumor but not the unrelated P 815 X2 mastocytoma

Cytogenetic analysis of tumors has revealed major chromosomal abnormalities appearing during the primary events in neoplastic disease and has identified karyotypic changes associated with the malignant evolution of tumors [2,4,11,13,15,16] In recent years both immunoresistant and immunogenic mutant sublines of tumors have been identified and this altered relationship with the syngeneic host has been correlated with changes in the expression of membrane antigens [5,8,9] In addition Friburg[17] has described the loss of chromosomal markers associated with the appearance of an immunoresistant tumor mutant which was capable of being transplanted across a histocompatibility barrier

In the present study we examined a spontaneous immunogenic mutant of the L5178Y lymphoma called the L5178Y/Manitoba (L5178Y/M) which is uniformly rejected when inoculated subcutaneously in the syngeneic DBA/2 host [10] We demonstrated in the experiments described below that this mutant bears both chromosomal and antigenic markers in common with its parent line as well as having acquired new distinguishing chromosomal markers

Materials and Methods

Animals

Four week-old to ten week-old inbred male or female mice of DBA/2 strain (Jackson Laboratories Bar Harbor Maine) were used throughout the study

Tumor Lines

The L5178Y lymphoma used in this study was obtained from Dr G A Fischer Yale University This lymphoma arose as a spontaneous neoplasm in an aged DBA/2 mouse in 1952[12] and was later adapted for growth in tissue culture [6] A mutant derived from this line (L5178Y/M) was obtained from Dr R Noble University of British Columbia but also originated in the laboratory of Dr Fischer The mutant line was first recognized in this laboratory in 1967 by its low virulence as measured by host sur

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vival The tumor has been kept in liquid nitrogen and the stock is replenished every one to two years

In Vitro Cultures

Tumors were cultured in Fischer s medium which was supplemented with 10% fetal calf serum penicillin and streptomycin (FFCS) The L5178Y was cultured at 2×10^4 per milliliter in 50 ml of FFCS medium and incubated at 37 C in sealed 250-ml flasks The tumor line was transferred into new medium every 48 hours to maintain the cells in a logarithmic growth rate It was necessary to transfer the L5178Y/M and its clones at the higher concentration of $7\,5\times10$ to 10^5 per milliliter to maintain an optimum growth rate

Tumor Cloning

Cloning was accomplished using a modification of the method of Chu and Fischer[3] Ten cells of the L5178Y/M line were seeded into 0 24% Agar Noble (Difco Laboratories Detroit Michigan) and cultured for ten days Isolated clones were chosen near the agar surface and extracted with a needle The cells were then injected into tissue culture medium and after seven to ten days growth the tumor cells were frozen and stored at −70 C The tumor clones were maintained in culture and restarted from frozen stock every six to eight weeks The three clones of the L5178Y/M utilized in this study—L5178Y/M 1 L5178Y M 2 and L5178Y/M 5—were recloned from the original isolates to ensure homogeneity

Chromosomal Analysis

Colcemid was added to the tumor cell cultures to produce a final concentration of 0 05μg/ml of medium Three hours later the cells were harvested following hypotonic treatment and fixed in ethanol acetic acid (3 1) fixative as described previously[1] Conventional air-dried preparations were used for chromosome analysis A minimum of 300 cells was examined for each tumor line

Tumor Growth Rates

Tumor cells were injected in a 0 1 ml volume in the shaved lower midback of six week-old to ten week old mice Tumor growth rates were determined macroscopically with Vernier calipers measuring two diameters of the tumor at right angles and the area of the tumor was expressed as the product of these two measurements The mean and standard error of the mean were calculated for groups of five to ten mice

Results

Chromosomal Analysis of the Tumor Lines

The karyotypic analysis of the L5178Y parent tumor line revealed that 46% of the cells had 41 chromosomes as compared to the normal mouse diploid chromosome complement of 40 (Figure 1) A small percentage of tetraploid cells was also identified In comparison 65% of the cells of the mutant L5178Y/M had 39 chromosomes Of the three clones isolated from the L5178Y/M clone 1 was diploid with 42% of its cells containing 40 chromosomes while clones 2 and 5 were hypotetraploid

The L5178Y had four marker chromosomes with the morphology of each as follows (Figure 2) number 1 chromosome was metacentric number 2 was an acrocentric chromosome with a distinguishable secondary constriction number 3 was an acrocentric chromosome most probably with an extended short arm and number 4 was the smallest acrocentric chromosome Both the L5178Y/M and the L5178Y/M clone 1 had three similar marker chromo

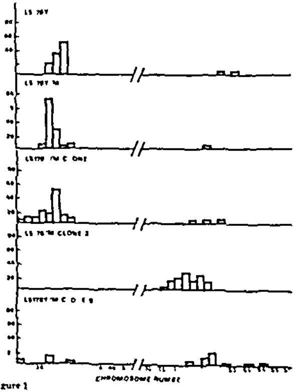

Figure 1
Chromosomal content of the L5178Y L5178Y/M and its clones Modal chromosome numbers are L5178Y 41 L5178Y/M [illegible] 39 L5178Y/M clone 1 40 L5178Y/M clone 2 [illegible] and L5178Y/M clone 5 79

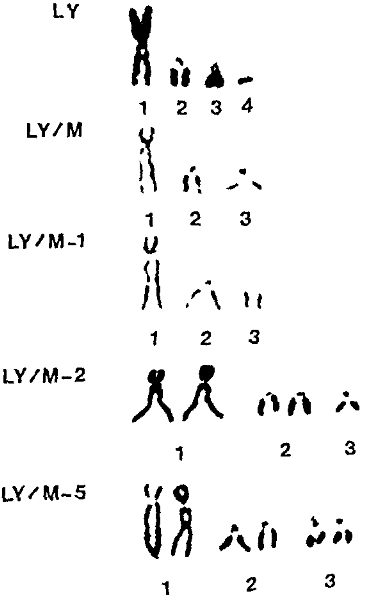

Figure 2
Chromosomal markers of the L5178Y (LY) L5178Y/M (LY/M) and its clones (LY/M 1 LY/M 2 and LY/M 5) Marker chromosome number 2 is common to both the parent and mutant lines including all the clones derived from the mutant although represented in pairs in the tetraploid clones The mutant lines all share the submetacentric chromosome marker number 1 which differs from the larger metacentric marker of the parent L5178Y

somes of which number 1 was submetacentric number 2 was an acrocentric chromosome with a distinguishable secondary constriction and number 3 was an acrocentric chromosome with a very prominent secondary constriction Marker chromosomes numbers 1 and 2 of the lines L5178Y/M clone 2 and clone 5 were similar to the L5178Y/M but in pairs while marker number 3 of L5178Y/M clone 2 which was similar to the other clones was present only as a single chromosome Marker chromosome number 2 was common to all the lines described here including the parent L5178Y

Tumor Growth in Syngeneic DBA/2 Mice Receiving the L5178Y L5178Y/M and Its Clones

Examination of the growth rates of the parent and mutant tumor clones also revealed unusual differences in the rejection patterns DBA/2 mice were challenged by subcutaneous inoculation in the shaved middorsal area with 10^7 viable tumor cells of the parent L5178Y line or the L5178Y/M and its clones 1 2 and 5 The parent L5178Y grew continuously and was uniformly lethal to the host All the L5178Y/M lines produced measurable tumors by day 5 reached a maximum size between days 7 and 11 and were completely rejected by day 19 (Figure 3) The L5178Y/M clone 1 grew more rapidly than all the other lines including the parent L5178Y achieving a maximum size which was four times that of the other L5178Y/M clones before rejection occurred Mice which were made immunodeficient by adult thymectomy lethal irradiation (900 rads) and bone marrow reconstitution were unable to reject the mutant tumor (unpublished data)

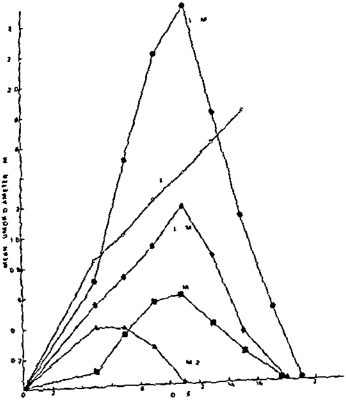

Figure 3
Growth rate of a subcutaneous injection of 10 tumor cells of L5178Y (LY) L5178Y M (LY/M) and the three clones (LY M 1 LY/M _ and LY/M 5) derived from the L5178Y/M The L5178Y/M lines caused detectable tumors in the mice by day 5 and were completely rejected by day 19 while the parent L5178Y grew progressively and killed all of the challenged mice

In Vitro *Growth Rates*

The *in vitro* growth rates of the L5178Y L5178Y/M and its clones were studied over a period of four weeks Tumor cells were taken from frozen stock and established in culture for two weeks until their growth was constant and exponential then the growth rates were calculated by the extrapolation method[1] over the following two week period All of the mutant tumor lines had a much slower growth rate than the parent L5178Y (Figure 4) A doubling time (dt) of 12 hours was observed for the L5178Y while the L5178Y/M had a dt of 20 hours L5178Y/M 5 was the slowest growing of the clones (dt = 26 hours) and clone 1 the fastest (dt = 19 hrs)

Immunologic Cross Reactivity Between the L5178Y and L5178Y/M

The specificity of the rejection of the L5178Y/M was assessed by immunizing DBA/2 mice with 5×10^6 mutant tumor cells and then challenging at the same site with the parent L5178Y line or two other DBA/2 tumors the P 815 X2 mastocytoma and the SL2 lymphoma Each was injected subcutaneously with a 10 cell inoculum 14 days after the L5178Y/M immunization and the growth and mortality rates followed (Figure 5) A significant slowing in L5178Y growth and a reduction in the mortality rate from 100% to 40% was observed in the L5178Y/M immune mice

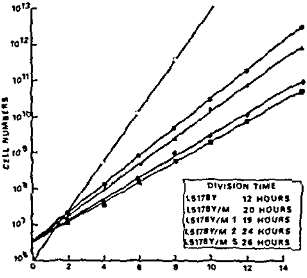

Figure 4
In vitro growth rate of the L5178Y (○) L5178Y/M (▲) and the L5178Y/M clones L5178Y/M 1 (●) L5178Y/M 2 (◆) and L5178Y/M 5 (■)

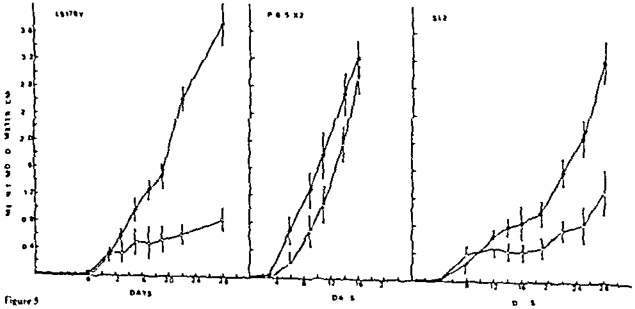

Figure 5
Specificity of the rejection of the L5178Y/M tumor Fourteen days after DBA/2 mice had rejected a subcutaneous injection of 5×10^6 mutant tumor cells a 10 cell inoculum of the parent L5178Y the P-815-X2 mastocytoma or SL2 lymphoma was administered at the same site and the tumor growth and mortality rate followed Both the L5178Y and SL2 lymphoma had significantly slowed growth rates in L5178Y/M immune mice (○) as compared to non immune controls (●) In addition the mortality rate was reduced from 100% in non immune mice to 40% and 31% for the L5178Y and SL2 respectively The P-815-X2 mastocytoma growth and mortality rate was unaffected by the immunization

while a completely unrelated tumor the P-815 X2 mastocytoma was not affected The SL2 lymphoma a tumor with a T-cell origin similar to the L5178Y lines also had a significantly reduced tumor growth rate and the mortality rate dropped from 100% in nonimmune mice to 51% in L5178Y/M immune mice

Discussion

Although the L5178Y/M has mutated enough to alter its fate in the syngeneic host it has retained both a chromosomal marker of the parent L5178Y and a common cell-surface antigen or antigens The chromosomal marker number 2 of the parent L5178Y (Figure 2) was found in all clones of the L5178Y/M as well as in all cells of the uncloned L5178Y/M that have been examined A shared antigen was demonstrated *in vivo* when it was shown that mice made immune to the L5178Y/M were able to reject or slow the growth of the parent line The observation that both a chromosomal marker and antigen are shared by the parent and mutant tumor lines establishes that the mutant tumor is not a contaminant but in fact is derived from the L5178Y The mutation however can be readily identified by major changes in the karyotype and tumor growth rates in addition to its altered *in vivo* fate The chromosomal constitution of the L5178Y/M was easily distinguished from the parent L5178Y by a larger chromosome complement as well as by two new markers the submetacentric marker number 1 and the acrocentric marker number 3 with the prominent secondary constriction

The *in vivo* and *in vitro* growth rates of the tumors varied markedly Both of the hypotetraploid clones of the L5178Y/M mutant had longer doubling times than the diploid mutant clone while the diploid mutant was slower growing than the parent L5178Y Although *in vivo* growth rates reflect both the host s ability to react to the tumor and its inherent growth properties it was of interest to note that the *in vivo* growth rates of the hypotetraploid mutants were again slower than that of the diploid mutant clone

These results indicate that the L5178Y/M lymphoma has mutated sufficiently to be identified by both its karyotypic markers and its altered relationship with the host It is not clear at this time however whether the chromosomal mutations are related to the enhanced immunogenicity of the L5178Y/M lymphoma

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References

1 Alexander P Mikulski M Differences in response of leukemic cells in tissue culture to nitrogen mustard and dimethyl myleran Biochem Pharmacol 5 275 283 1961

2 Atkin NB Baker HC Chromosome abnormalities as primary events in human malignant disease Evidence from marker chromosomes J Natl Cancer Inst 36 539 5 7 1966

3 Chu MY Fischer GA The incorporation of H-cytosine arabidnomide and its effect on murine leukemic cells (L5178Y) Biochem Pharmacol 17 753-761 1968

4 Dofuku P Biedler JL Spengler BA Old LJ Trisomy of chromosome 15 in spontaneous leukemia of AKR mice Proc Natl Acad Sci USA 72 1515 1517 1975

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6 Fischer GA Studies of the culture of leukemic cells in vitro Ann NY Acad Sci 76 673 1958

7 Friberg S Comparison of an immunoresistant and immunosusceptible ascites subline from murine tumor TA3 I Transplantability morphology and some physicochemical characteristics J Natl Cancer Inst 48 1463-1476 1972

8 Friberg S Comparison of an immunoresistant and an immunosuspectible ascites subline from murine tumor TA3 II Immunosensitivity and antibody-binding capacity in vitro and immunogenicity in allogeneic mice J Natl Cancer Inst 48 1477 1489 1972

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Cytologic Effects of Thiotepa and Adriamycin on Normal Canine Urothelium

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John Sall, M.D.

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Major observations in the experimental animals included cellular enlargement, bizarre cell shapes, cytoplasmic vacuolization, nuclear and nucleolar enlargement, multinucleation, pyknosis, karyorrhexis and infiltration by neutrophilic leukocytes. In some animals reversion to a normal cytologic pattern occurred in the ten week period. The application of these observations to human urinary cytopathology is discussed.

Cytologic study is an essential technique for the follow-up evaluation of patients undergoing treatment for transitional cell carcinoma of the urinary tract. Increasing use of chemotherapeutic agents in the treatment of these patients[7,17,18] introduces complications into cytologic interpretations inasmuch as the agents themselves produce morphologic alterations in the epithelial cells. Thus it becomes essential to identify the precise changes that can be attributed to these drugs so that they can be distinguished from features that may represent persistent or recurrent malignancy. It is difficult to control and separate these cellular features in patients.[10] The current studies were carried out in normal experimental animals, in which chemotherapeutic drug effects could be carefully produced and observed under controlled conditions. Female dogs underwent a treatment regimen with triethylene thiophosphoramide (Thiotepa), an alkylating agent structurally related to nitrogen mustard, or alternatively with doxorubicin hydrochloride (Adriamycin), a cytotoxic tumor antibiotic isolated from *Streptomyces peucetius* var. *caesius*.[1] The cytologic effects of these drugs on normal canine urothelium were studied by light microscopy.

Materials and Methods

Fifteen female dogs of similar size were weighed, anesthetized with intravenous sodium pentobarbitol and placed in a modified lithotomy position. Perivulvar skin was shaved and cleansed with pHisoHex (Winthrop Laboratories, New York, New York). Using a number 16-18 Foley catheter, each dog was catheterized and bladder urine obtained (20 to 100

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while a completely unrelated tumor the P 815-X2 mastocytoma was not affected The SL2 lymphoma a tumor with a T-cell origin similar to the L5178Y lines also had a significantly reduced tumor growth rate and the mortality rate dropped from 100% in nonimmune mice to 51% in L5178Y/M immune mice

Discussion

Although the L5178Y/M has mutated enough to alter its fate in the syngeneic host it has retained both a chromosomal marker of the parent L5178Y and a common cell surface antigen or antigens The chromosomal marker number 2 of the parent L5178Y (Figure 2) was found in all clones of the L5178Y/M as well as in all cells of the uncloned L5178Y/M that have been examined A shared antigen was demonstrated *in vivo* when it was shown that mice made immune to the L5178Y/M were able to reject or slow the growth of the parent line The observation that both a chromosomal marker and antigen are shared by the parent and mutant tumor lines establishes that the mutant tumor is not a contaminant but in fact is derived from the L5178Y The mutation however can be readily identified by major changes in the karyotype and tumor growth rates in addition to its altered *in vivo* fate The chromosomal constitution of the L5178Y/M was easily distinguished from the parent L5178Y by a larger chromosome complement as well as by two new markers the submetacentric marker number 1 and the acrocentric marker number 3 with the prominent secondary constriction

The *in vivo* and *in vitro* growth rates of the tumors varied markedly Both of the hypotetraploid clones of the L5178Y/M mutant had longer doubling times than the diploid mutant clone while the diploid mutant was slower growing than the parent L5178Y Although *in vivo* growth rates reflect both the host s ability to react to the tumor and its inherent growth properties it was of interest to note that the *in vivo* growth rates of the hypotetraploid mutants were again slower than that of the diploid mutant clone

These results indicate that the L5178Y/M lymphoma has mutated sufficiently to be identified by both its karyotypic markers and its altered relationship with the host It is not clear at this time however whether the chromosomal mutations are related to the enhanced immunogenicity of the L5178Y/M lymphoma

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References

1 Alexander P Mikulski M Differences in response of leukemic cells in tissue culture to nitrogen mustard and dimethyl myleran *Biochem Pharmacol* 5 275-283 1961

2 Atkin NB Baker HC Chromosome abnormalities as primary events in human malignant disease Evidence from marker chromosomes *J Natl Cancer Inst* 36 539 557 1966

3 Chu MY Fischer GA The incorporation of ³H-cytosine arabidnomide and its effect on murine leukemic cells (L5178Y) *Biochem Pharmacol* 17 753 761 1968

4 Dofuku P Biedler JL Spengler BA Old LJ Trisomy of chromosome 15 in spontaneous leukemia of AKR mice *Proc Natl Acad Sci USA* 72 1515 1517 1975

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ml) A sterile disposable 60-ml syringe filled with 50 to 60 ml of normal saline was attached to the catheter and the saline injected into and then withdrawn from the bladder at least twice If washings were turbid in appearance a second barbotage was done with a new volume of normal saline

The cathetherized urine specimens and bladder washings were refrigerated for a period of not more than two hours They were then processed as follows an equal volume of 50% ethyl alcohol was added to each sample and a minimum fixation time of 30 minutes allowed Several 8µm Millipore filter preparations (Millipore Corporation Rosemont Illinois) were made from each sample and stained according to a modified Papanicolaou staining technique *

A 24 French Brown Buerguer cystoscope was used to inspect the bladder mucosa In one dog it was necessary to perform a meatotomy to allow passage of the cystoscope A cold punch biopsy forceps was used to obtain biopsy specimens from the trigone and posterior bladder wall and these specimens were fixed in 10% neutral buffered formalin A dissecting microscope was used to align biopsy material and the tissue was embedded in 2% agarose in distilled water The samples were then oriented in paraffin and hematoxylin-and-eosin sections were prepared

Five dogs had the Foley catheter reinserted and 30 mg of Thiotepa (Lederle Laboratories Pearl River New York) in 30 ml saline* was instilled into the bladder and retained for a period of one to two hours At the completion of this period the bladder was drained and the catheter was removed Five other dogs received Adriamycin (Adria Laboratories Inc Wilmington Delaware) intravenously in the concentration of 1 mg/kg of body weight Five control dogs had the Foley catheter reinserted and normal saline instilled into the bladder and retained for a period of one to two hours The bladders were drained at the completion of this period and the catheters removed

All dogs received repeat treatment with the drugs or saline at weekly intervals for ten weeks Blood profiles were monitored weekly by performing complete blood counts and platelet counts and additionally in dogs receiving Adriamycin by determining serum glutamic oxaloacetic transaminase (SGOT) and creatine phosphokinase (CPK) All dogs were cystoscoped and biopsy specimens obtained between weeks three and five following commencement of drug administration and again at weeks ten to 12

Week ten was the final administration of drugs in all dogs At that time a lethal dose of sodium pentobarbitol was injected and autopsies were conducted Autopsy tissue was obtained from bladder fundus bladder trigone and renal pelves Representative sections were also taken from heart trachea lung pancreas spleen liver and alimentary mucosa

Results

The results are summarized in Table I Most pretreatment urine and bladder washing samples had small numbers of urothelial cells Pretreatment biopsy specimens showed bladder epithelium one to four cell layers thick

Thiotepa Experiments

In bladder washing samples hypercellularity was noted in weeks two to three following initiation of drug treatment (Figure 1) Cellular abnormalities began to appear in occasional cells at that time The features were nuclear and cellular enlargement prominent nucleoli and cytoplasmic vacuolization During weeks three to six in all treated dogs the cellular changes were the most bizarre and the most numerous (Figures 2A and 2B) In addition to the above-mentioned abnormalities multinucleation and leukocytic infiltration into urothelial cells were prominent features (Figures 3A and 3B) Beginning in weeks six or seven the cellular pattern was that of degeneration pyknosis and karyorrhexis were common Two dogs showed a moderate inflammatory response in urine and bladder washings in week two

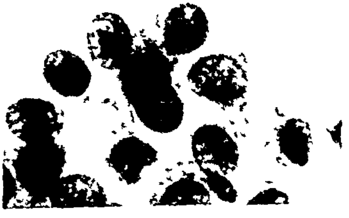

Figure 1
Bladder washing week two Thiotepa treatment Hypercellularity and slight nuclear enlargement (Papanicolaou stain × 400)

The dosage of Thiotepa was determined by National Bladder Cancer Collaborative Group A (NBCCGA) protocol 3

Table 1 *Summary of Cytologic Effects of Thiotepa and Adriamycin*

Sample	*Saline control*	*Thiotepa*	Adriamycin
Biopsy week 3 5	1-4 cell layers	1-4 cell layers occasional cells with *nuclear and nucleolar enlargement*	1-4 cell layers 1 sample with 6-10 cell layers *1 sample with nuclear and* nucleolar enlargement throughout epithelium with occasional cytoplasmic vacuolization and leukocytic infiltration
Biopsy week 10-12	2-4 cell layers	1 2 cell layers (4 dogs) 2 5 cell layers (1 dog)	1-6 cell layers prominent nuclear and nucleolar enlargement
Bladder washing week 2 3	No changes many degenerated cells	Hypercellularity occasional cells with nuclear and nucleolar enlargement cytoplasmic vacuolization	Increase in cells
Bladder washing week 3 5	No changes many degenerated cells	Cellular changes numerous multinucleation and leukocytic infiltration prominent	Increase in cells occasional cells with slight to moderate nuclear and nucleolar enlargement
Bladder washing week 5-6	Increase in cells	Pyknosis karyorrhexis	Slight to moderate nuclear and nucleolar enlargement (4 dogs) marked nuclear and nucleolar enlargement multinucleation cytoplasmic vacuolization leukocytic infiltration (1 dog)
Bladder washing week 8 10	Occasional cells with slight nuclear enlargement	Pyknosis karyorrhexis	Pyknosis anucleate urothelial cells repair (2 dogs)
Autopsy bladder week 10 1	2-6 cell layers (4 dogs) 4 10 cell layers (1 dog) *leukocytic infiltration (1 dog)*	3 10 cell layers (4 dogs) 3-5 cell layers (1 dog) many individual cells with nuclear enlargement and prominent and multiple nucleoli and leukocytic infiltration	6-10 cell layers (3 dogs) 3 5 cell layers (dogs) individual cells with nuclear enlargement and prominent nucleoli leukocytic infiltration

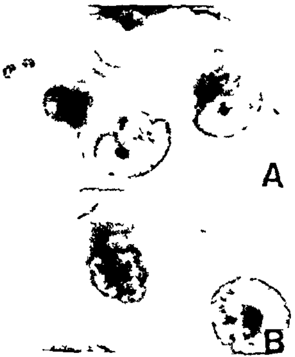

Figure 2
Bladder washings week three and four Thiotepa treatment (A B) [illegible] nuclear enlargement [illegible] nuclear shape and chromatin [illegible] × 450

Biopsy specimens taken during the third to fifth week following commencement of drug treatment showed bladder epithelium one to four cell layers thick occasional cells with nuclear and nucleolar enlargement were noted in some samples Biopsies at week ten demonstrated one to two cell layers of epithelium in four dogs and two to five cell layers of epithelium in one dog No individual cell atypia was noted in biopsy material at week ten

Autopsy bladders of four out of five dogs treated with Thiotepa showed bladder epithelium up to ten cell layers in thickness with many individual cells showing nuclear enlargement and prominent and multiple nucleoli (Figure 4) The fifth dog showed no abnormality in bladder epithelium and typical epithelium was three to five cell layers in thickness

One of the dogs that received Thiotepa developed moderate leukopenia and thrombocytopenia during the ninth week The other animals showed no significant hematologic abnormalities

Adriamycin Experiments

In bladder washings hypercellularity of urothelial cells was seen in weeks five to six following initial administration of the drug Slight to moderate nuclear and cellular enlargement was occasionally pres

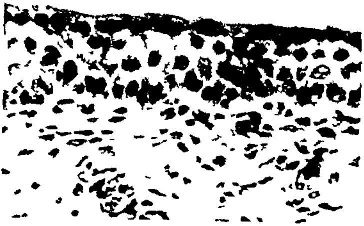

Figure 9
Autopsy bladder specimen week 11 saline treatment (hematoxylin and eosin × 160)

Discussion

Cellular changes associated with various types of *chemotherapeutic agents have* been previously explored to a variable extent Busulfan associated atypia has been thoroughly reviewed and described by Koss et al[9] and Feingold and Koss [4] These changes have been observed in many organs of the body especially the cervix urinary bladder and lung but sometimes in the pancreas kidney liver esophagus bone marrow and endothelium [4 9 11] Variously these changes have been described as cellular and nuclear *enlargement nuclear hyperchromasia abnormal* giant cells atypical hyperplasia atypical cells dyskaryosis dysplasia and cells resembling carcinoma *in situ* Similar changes have been described in voided urine samples from patients undergoing treatment with Cytoxan [5 8 15 19]

Because of such observations we expected to see evidence at autopsy of hyperplasia or cellular atypia in organs other than the urinary tract particularly in the Adriamycin treated animals inasmuch as this drug was administered systemically However the busulfan-associated atypia described by Koss et al was seen in autopsy material from patients under long-term (four months to four years) drug therapy [9] Probably the period of drug administration in our experiments was too short for these systemic effects to develop

Documentation of Thiotepa-associated atypia is not as abundant Following Thiotepa treatment of bladder cancer changes noted varied from 'higher class Papanicolaou smears on urine in a patient with *no cystoscopic* evidence of tumor[3] to a few solitary cells [autopsy bladder] [showing] dyskaryotic changes [1] Interestingly in 1958 Moore observed various degrees of cellular changes such as swelling vacuolization pyknotic nuclei and abnormal giant cells in serial biopsies taken from tumor nodules in patients who had advanced metastatic breast cancer and were being treated with Thiotepa [12]

More recent reports are contradictory Murphy et al deny cellular changes specific for Thiotepa[13-14] while de Voogt et al attribute to Thiotepa findings identical to those reported here i e cellular and nuclear enlargement enlarged nucleoli abnormal cell shapes multinucleation and vacuolization [2]

All of the drug related *features that have been* previously described except hyperchromasia were observed in urothelial cells in our experiments with Thiotepa and Adriamycin Hyperchromasia was *not observed* in *any* nuclei in *cells* from catheterized urine and bladder washings from our experimental animals This becomes of special importance as one attempts to evaluate cytology specimens in patients with bladder cancer who are undergoing chemotherapy The demonstration of true hyperchromasia in such samples should raise suspicions that such patients have persistent tumor rather than suggest that the hyperchromasia can be attributed to drug effect On the other hand when cytologic changes similar to those observed in our experiments are encountered in patient specimens they can be attributed to drug therapy and rather confidently distinguished *from neoplastic changes*

References

1 Bruce DW Edgcomb JH Pancytopenia and generalized sepsis following the treatment of cancer of the bladder with instillations of *triethylene thiophosphoramide* J Urol 97 482-485 1967

2 DeVoogt HJ Rathert P Beyer Boon ME Urinary Cytology Berlin Heidelberg Springer Verlag 1977 pp 40-41 168

3 Drew JE Marshal VF *The effects of topical Thiotepa on the* recurrence rate of superficial bladder cancers J Urol 99 740-743 1968

4 Feingold M Koss LG Effects of long-term administration of busulfan Arch Intern Med 124 66-71 1969

5 Forni A Koss LG Geller W Cytological study of the effect of cyclophosphamide on the epithelium of the urinary bladder in man Cancer 17 1348-1355 1964

6 Gill GW Exfoliative Cytology Application Report -4 Bedford Massachusetts Millipore Corporation 1969

7 Jones HC Swinney J Thiotepa in the treatment of tumors of the bladder Lancet 2 615-618 1961

8 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Philadelphia JP Lippincott 1979 pp 738-743

9 Koss LG Melamed MR Mayer K The effect of busulfan on human epithelia Am J Clin Pathol 44 385-397 1965

10 Loveless KJ The effects of radiation upon the cytology of benign and malignant bladder epithelia Acta Cytol 17 355 360 19 3

11 Min KW Gyorkey F Interstitial pulmonary fibrosis atypical epithelial changes and bronchiolar cell carcinoma following busulfan therapy Cancer 22 10 7 1032 1968

12 Moore GE Clinical experience with triethylene thiophosphoramide with special reference to carcinoma of the breast Ann NY Acad Sci 68 1074 1090 1958

13 Murphy WM Soloway MS Lin CJ Morphologic effects of thio TEPA on mammalian urothelium Changes in abnormal cells Acta Cytol 22 550-554 1978

14 Murphy WM Soloway MS Lin CJ Morphologic effects of thio TEPA on mouse urothelium Acta Cytol 1 701 704 1977

15 Slavin RE Millan JC Mullins GM Pathology of high dose intermittent cyclophosphamide therapy Hum Pathol 6 693-709 1975

16 Tan C Etcubanas E Wollner N Rosen G Gilladoga A Showel J Murphy ML Krakoff IH Adriamycin An antitumor antibiotic in the treatment of neoplastic diseases Cancer 32 9 17 1973

17 Veenema RJ Dean AL Jr Roberts M Fingerhut B Chowhury BK Tarossoly H Bladder carcinoma treated by direct instillation of Thiotepa J Urol 88 60-63 196

18 Veenema RJ Romas NA Fingerhut B Chemotherapy for bladder cancer Urology 3 135 139 1974

19 Voutsa NG Melamed MR Cytology of in situ carcinoma of the human urinary bladder Cancer 16 1307 1316 1963

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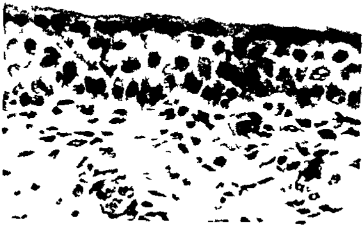

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17 Veenema RJ Dean AL Jr Roberts M Fingerhut B Chowhury BK Tarossoly H Bladder carcinoma treated by direct instillation of Thiotepa J Urol 88 60-63 1962

18 Veenema RJ Romas NA Fingerhut B Chemotherapy for bladder cancer Urology 3 135 139 1974

19 Voutsa NG Melamed MR Cytology of *in situ* carcinoma of the human urinary bladder Cancer 16 1307 1316 1963

16th Annual Cytology Workshop-Seminar

The 16th Annual Cytology Workshop Seminar sponsored by the California Association of Cytotechnologists will be held June 6–8 1980 at the Grand Hotel Anaheim California This meeting has been consistently regarded as one of the most outstanding programs of its kind traditionally inviting prominent figures in the field of cytology as guest faculty

The speakers and workshop directors for this meeting will be Gary Gill C T (ASCP) C M I A C Marion Danos Holmquist C T (ASCP) C M I A C and Catherine M Keebler C T (ASCP) C F I A C Topics for discussion will include the principles and practice of cytopreparation the female genital tract current cytologic criteria and gynecologic cytology diagnostic problems in the patient over 55

Additional information about this program may be obtained from CAC Workshop Seminar 10480 Gregory Circle Cypress California 90630 (phone 714/828-0621 or 213/795 3815)

In Vitro *Cell Changes in Laser-Exposed Tissue*

Nancy A Shepanek, C T (ASCP)
Barbara J Kaplan, C T (ASCP),
C M I A C
Duane Townsend, M D

Tissue cultures of WI 38 human embryonic lung were exposed to CO_2 laser irradiation while others were maintained unexposed as controls In the test cultures the target area demonstrated cell dehydration and distortion consisting of nuclear and cellular elongation and cytoplasmic fragmentation Regeneration of the cultures was gradual and incomplete

Since its introduction into gynecologic surgery in 1964 by McGuffi (cited by Mester[4]) the laser has been used to treat adenosis condyloma acuminatum herpes simplex erosion[3] and intraepithelial neoplasia [2]

According to Stellar [5] the laser produces a characteristic picture in the epithelium at the point of impact affecting three layers of cells The outer layer where vaporization occurs shows carbon deposition In the middle layer, vaporization is subtotal where fluid is vaporized pseudotubules with holes are surrounded by damaged cellular material The deepest layer is a thin sheet of edematous cells The depth of all three measures 100µm to 500µm [6] Tissue cultures exposed to laser irradiation have shown intra and extracellular changes including chromosome abnormalities and nucleolar and extranucleolar lesions [1]

Cytologic changes following laser treatment were described by Holmquist et al[2] and included coagulation and lengthwise distortion of the cytoplasm of columnar and fibrocytic cell with elongation of the nucleoli Squamous cells in the area were less altered Similar changes were seen in cells from tissue samples from the target region The tissues also demonstrated stenosed blood vessels According to Holmquist et al [2] these cells may represent dehydration of adjacent tissue in response to vaporization of the target area

Our purpose was to document the effect of the laser on cells in culture

Materials and Methods

We obtained 24 tissue cultures of WI 38 human embryonic lung fibroblasts grown in flasks with 90% Hank s Minimal Essential Medium (HMEM) supplemented with 10% fetal bovine serum (Flow Laboratories Los Angeles California) The cells were attached to floating glass coverslips The cultures were separated into two groups six cultures were exposed to the CO_2 laser beam at 5 w amperage and six at 10 w amperage for 1/10 second The caps were removed from the flasks during the exposure and the beam was pointed directly at the cells through the opening Twelve control cultures were set up and maintained without treatment

One 5 w culture one 10 w culture and one control were harvested at each of the following numbers of hours after treatment 2 5 23 30 97 and 100 At each harvest the coverslips that carried the cultures were removed from the flasks placed in 95% ethyl alcohol and stained by the Papanicolaou technique as modified for our laboratory They were then placed

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on plain glass slides with mounting medium and coverslipped

Results

At two hours after treatment both the 5 w and 10 w cultures showed concentric circles of cells in the target area with each circle displaying a different effect The area was 4 mm in diameter (Figure 1) The center *contained cell debris and few intact cells with* smudged nuclear detail and wavy bubbly cytoplasm This central area region 1 (Figure 2) stained pink with the Papanicolaou stain in contrast to the blue color of the other regions The area of destruction was consistently larger in the 10 w cultures than *in the 5 w cultures but the cell changes within the* different regions were similar with the descriptions reflecting what was seen in both the 5 w and 10 w cultures

The cells in region 2 (Figure 3) showed clearer nuclear detail but the cytoplasm was still bland and wavy *These cells looked dry Region 3 (Figure 4)* consisted of a partially cleared space which contained cell fragments with elongated nuclei The cell remnants were thinner and smaller than normal *in vitro* fibroblasts Cells in region 4 (Figure 5) appeared to be normal

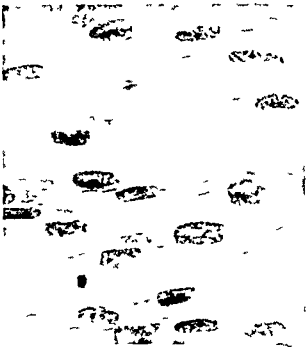

Figure 2
Ten watt culture two hours after laser showing the intact cells in region 1 Notice the smudged nuclear chromatin and wavy cytoplasm (× 512)

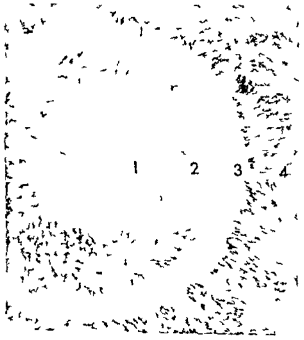

Figure 1
Ten watt culture two hours after laser showing regions 1 2 3 and 4 (× 16)

Figure 3
Ten watt culture two hours after laser showing bubbly or bland wavy cytoplasm with bubbly or grainy nuclei in region 2 (× 512)

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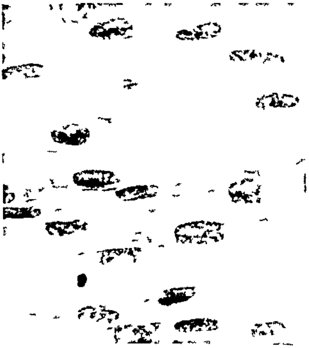

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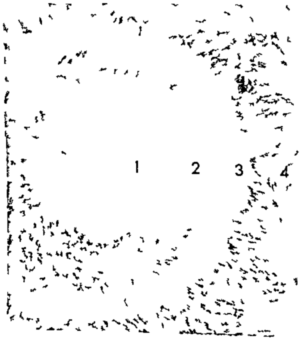
Ten watt culture two hours after laser showing the intact cells in region 1 Notice the smudged nuclear chromatin and wavy cytoplasm (× 512)



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Ten watt culture two hours after laser showing bubbly or bland wavy cytoplasm with bubbly or grainy nuclei in region 2 (× 512)

Cell features and growth patterns five hours after treatment resembled those at two hours

After 23 hours normal cells were seen in region 2 There was no clear zone in region 3 in the 5 w culture *A clear zone appeared* in the 10 w culture but it was narrower than the clear zone in the earlier 10 w harvest The number of elongated cells had diminished and normal cells were stretched across this region from the *normal to the damaged tissue*

At 97 hours normal cells were scattered within region 1 where they grew atop the cell debris No zones could be distinguished by 100 hours after treatment Karyorrhexis and other features consistent *with cell death* were apparent

The control cultures showed a normal pattern of growth with mitoses beginning at 24 hours and continuing through this experiment

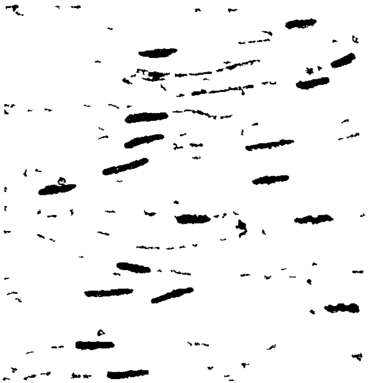

Figure 4
The elongated cells in region 3 (× 512)

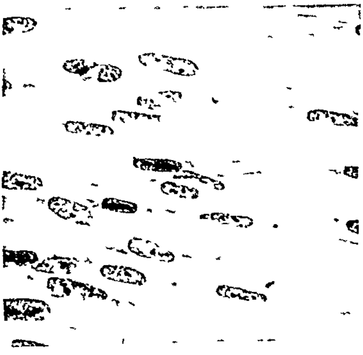

Figure 5
Normal fibroblasts at the periphery of the affected area region 4 (× 512)

Discussion

According to Holmquist et al [2] one of the benefits of laser therapy for vaginal and cervical lesions is that *damage to adjacent tissue is minimal* We believe this was confirmed in culture where the affected area was demarcated from the normal

Regrowth appeared within region 2 surrounding the target 24 hours after treatment Active *in vivo* proliferation of squamous epithelium has been recognized at about ten days after treatment [2] Regeneration was incomplete in the treated cultures through *the 100th hour*

At the 100th hour the control cultures showed few signs of cell death while some treated cultures showed many This may have been due to contamination the treated cultures were uncapped during *laser irradiation However we did not test for contaminants* The cultures were not fed nor were they pH adjusted during the experiment and this condition may have contributed to cell demise since toxins from the dead laser-affected cells were probably present

Acknowledgments

We appreciate the suggestions of N D Holmquist regarding this manuscript

References

1 Berns MW Round DE Laser microbeam studies on tissue culture cells Ann NY Acad Sci 168 550-563 1969

2 Holmquist ND Bellina JH Danos ML Vaginal and cervical cy*tologic changes* following laser treatment Acta Cytol 20 290-294 1976

3 Kaplan I Goldman J Ger R *The treatment of erosions of the* uterine cervix by means of the CO_2 laser Obstet Gynecol 41 795-796 1973

4 Mester E Korenyi Both A Kovacs I Spiry T Tisza S Laser in the locoregional treatment of tumors Panminerva Med 17 229-234 1975

5 Stellar S The carbon dioxide laser in experimental and clinical surgery *for neoplasms* Panminerva Med 17 223-228 1974

Exfoliative Cytology in Experimental Infections with Schistosoma haematobium

Jerry A Moore M S
Robert E Kuntz Ph D
Tao-cheng Huang B S

An association between carcinoma of the urinary bladder and Schistosoma haematobium *infection one of the major schistosomiases of humans has led to extensive investigations of this disease at the laboratory level It has been demonstrated that the* S haematobium *capuchin monkey* (Cebus apella) *combination offers an excellent model for multidisciplinary in depth observations Exfoliative cytology developed as one of several approaches used to follow general pathobiology of the urogenital system has presented a more sophisticated means of studying vesical schistosomiasis and associated host parasite relationships*

The schistosomiases (bilharziases) are responsible for various disease conditions and critical morbidity in humans in many areas where different species of *Schistosoma* are endemic Extensive investigation at the laboratory level has elucidated much of the biology associated with schistosome infections thus providing a better understanding of human disease processes Conventional small laboratory animals have served as satisfactory models for most multidisciplinary work in schistosomiasis However the demonstration of a probable relationship between bladder cancer and *Schistosoma haematobium* infection in nonhuman primates[3 9] has necessitated their use for these studies as well as the development of technics for more critical monitoring of schistosome infection [11]

Fecal and urine examinations have been employed as standard technics in human survey type studies to detect the presence of schistosome eggs diagnose suspected infection and determine the efficacy of drug treatment In laboratory animals this procedure is used to establish patency dates and host parasite relationships However to our knowledge urinary or exfoliative cytology has not been utilized in evaluating the status of bladder pathology or in following the evolution of urogenital system involvement in *S haematobium* infections This report describes the use of exfoliative cytology as a means of attempting to judge the status of urogenital pathology in nonhuman primates experimentally infected with *S haematobium* (Iran strain)

Materials and Methods

In the past 14 years 18 species of nonhuman primates have been used for extensive experimental schistosomiasis investigations in this laboratory Details on procurement and handling of nonhuman primates and application of materials in multidisciplinary investigations have been described elsewhere [4 10 2 13] *Bulinus truncatus* the snail host for *S haematobium* was obtained from endemic areas and primates from import dealers Based upon experience the capuchin monkey (*Cebus apella*)-*Schistosoma haematobium* system was chosen as the most suitable combination for parasitologic expression of the disease Counted numbers of cercariae (the infective stage of schistosomes) were placed on shaved

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This study was supported by the United States Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases National Institutes of Health United States Public Health Service grant CA 13208 grant AICA 14999 from the National Cancer Institute and the National Institute for Allergy and Infectious Diseases and grant CA 16378 from the National Bladder Cancer Project

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and cleansed abdominal skin to allow a natural means of penetration and infection Infected hosts were held in individual cages equipped with fecal and urine catchpans for collection of excreta samples to be processed and examined by customary technics

Procurement of good-quality urine necessitated the restraint of test subjects and the use of pediatric urine collection bags Capuchin monkeys under light sedation (ketamine hydrochloride Ketaset Bristol Labs Syracuse New York) were transferred from holding cages to medium-sized restraint chairs (BRS Foringer Beltsville Maryland) They readily adapted to handling and to bags secured by cords over the external genitalia Urine production varied but as a rule 8 to 15 ml of urine was collected in two to five hours

Urine bag contents were poured into a Millipore filtering and suction apparatus for the collection of cellular elements on a 5μ Nucleopore membrane (Wallabs Inc Fairfax California) The membrane was fixed in 95% ethyl alcohol and stained by the *Gill modification*[8] *of the* Papanicolaou technic [14] employing stains E-65 and OG-6 (Harleco Gibbstown New Jersey) After dehydration in graded alcohols and clearing in xylene *the membrane was cover* slipped with permount

Tissue samples for histopathologic evaluations were fixed in 10% buffered formalin sectioned at 5μ and stained in hematoxylin and eosin

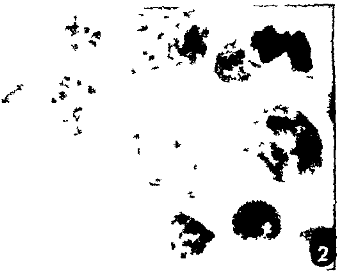
Results

A demand for in-depth studies of animals infected with *S haematobium* has led to the development of technics which allow evaluation of host parasite relationships particularly the effects of infection on the urogenital system Examination of numerous urine samples from capuchin monkeys which provided a model system for investigations in *S haematobium* infection has revealed that the onset of pathologic involvement of the urogenital system as well as general evolution of lesions may be followed by exfoliative cytology

Broad categories (i e negative suspicious and positive) were arbitrarily selected to indicate urothelial status Exfoliated cells ranging from normal (Figure 1) in control subjects and early prepatent infections to various stages of pathologic involvement (Figures 2 to 4) indicated by variations in cell numbers and morphologic features provided patterns which could be correlated with various stages in the development of the disease (Figures 6 to 8) In general the criteria for judging cytoplasmic and nuclear alterations (cellular pleomorphism modified cytoplasm enlarged and irregular nuclei cohesiveness of cells hyperchromicity and altered nuclear cytoplasmic ratios) were those described by Papanicolaou [14]

Patency was indicated by the presence of *S haematobium* eggs in urine at 65 to 100 days after exposure (Figure 5) Hypercellularity occurred at six to eight months after infection followed by hyperchromasia in transitional cells and loss of cohesiveness

Figure 1
Normal exfoliated transitional cells in urine of control capuchin monkey (*Cebus apella*) (Gill modification of Papanicolaou stain approximately × 100)



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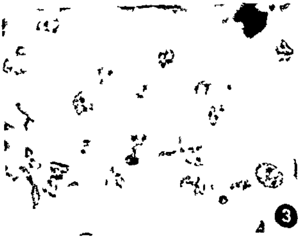

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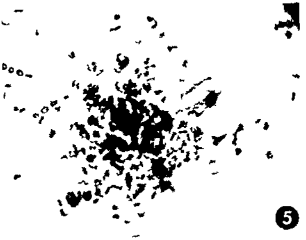

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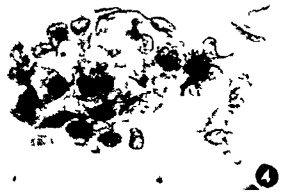
S haematobium egg with aggregation of inflammatory cells attached to surface as passed with urine from capuchin monkey (Gill modification of Papanicolaou stain approximately × 450)



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Clumps of transitional cells characterized by *anisonucleosis* hyperchromaticity and vacuolation (male capuchin approximately 30 months after infection Gill modification of Papanicolaou stain approximately × 450)

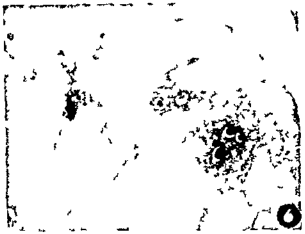

Figure 6
Bladder wall section showing proliferation and infolding of transitional cells (male capuchin monkey killed 13 months after infection hematoxylin and eosin approximately × 10)

indicating suspicious involvement of the urothelium At this stage egg passage was usually low (8 to 10 eggs/10 ml urine) There were few *polymorphonu*clear cells few lymphocytes and occasional plasma cells A designation of suspicious was changed to positive as cells in the urine became *less cohesive and* exhibited slight atypia hyperchromicity became more apparent and there was chromatic clumping with reduced cytoplasm

In capuchin monkeys followed for approximately four years it was determined by a combination of approaches (i e urine cytology X ray cystotomy and necropsy examinations) that macroscopic papillary bladder lesions persisted for 25 to 40 months in hosts subjected to a single schistosome infection In some hosts these technics also indicated general lesion regression at 18 to 36 months after infection with lymphocytes some giant cells and plasma cell showers in later stages Host parasite relationships with regard to lesions and urinary cytology were and cleansed abdominal skin to allow a natural means of penetration and infection Infected hosts were held in individual cages equipped with fecal and urine catchpans for collection of excreta samples to be processed and examined by customary technics

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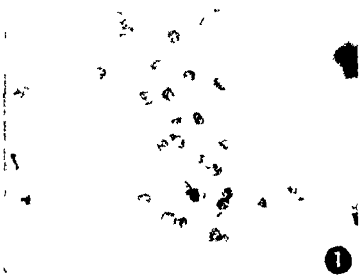

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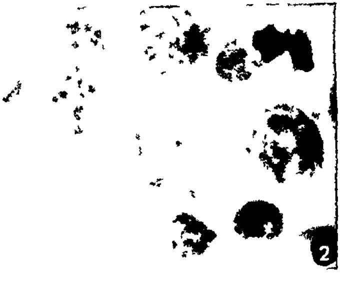

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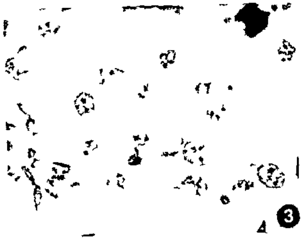

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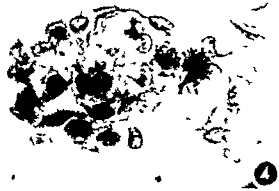

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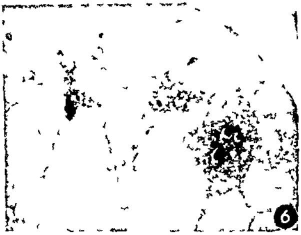

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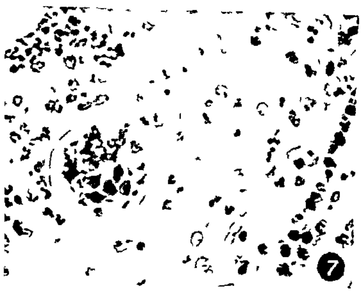

igure 7
ection of bladder showing abnormal transitional cells and inflam
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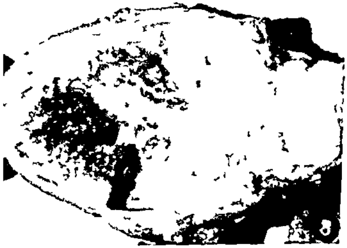

gure 8
oss bladder lesions *(multiple papillary transitional* cell carci
mas accompanied by squamous metaplasia) responsible for
sitive urine cytology evaluations prior to necropsy of infected
st *(same host as in Figure 7* approximately natural size)

haracterized by marked variations when animals re-ived challenge or multiple schistosome infections

In one project 72 capuchin monkeys (40 infected controls) were followed by urinary cytology at ast once a month for four years Cellular elements the urine of 16 of 40 infected hosts indicated thologic involvement of the urogenital system he presence of macrolesions was confirmed for most of these subjects by X ray cystotomy and histopathologic evaluations of biopsies In one instance no macroscopic bladder lesions were evident at necropsy in a host judged positive by urine cytology but small lesions were detected in sections of one ureter

An overall evaluation of urinary cytology as an acceptable technic for monitoring pathologic involvement of the urinary bladder in capuchin monkeys and African baboons (*Papio cynocephalus*) revealed a sensitivity greater than 85%

Discussion

An enormous literature on urinary cytology has been generated since 1945 when Papanicolaou and Marshall [15] described the use of urinary sediments as a diagnostic procedure for the detection of urinary tract cancer Although there has been much controversy regarding the efficacy and sensitivity of exfoliative cytology as a means of detecting urothelial alterations this procedure is used extensively for the study of multifocal pathology in the urogenital system However it has found only limited application in the hands of pathologists and parasitologists concerned with the pathobiology of schistosome disease even though *S haematobium* is recognized as an agent which drastically affects the urinary bladder

Dimmette et al[5] examined the urinary sediment of people in a schistosome-endemic area in Egypt to determine the usefulness of exfoliative cytology in a study of vesical schistosomiasis and although difficulties were encountered found a high correlation with bladder cancer Employing exfoliative cytology Elwi et al[7] found a high correlation with clinical and cystoscopic examination in cancer diagnosis in schistosome infected patients and stated that urinary cytology was especially useful in differentiating schistosome ulceration of the urinary bladder from malignancies El Bolkainy et al[8] reviewed the works of other investigators in Egypt and studied an additional long series of schistosome infected patients to further evaluate the accuracy of cytologic diagnoses based upon correlations with histopathologic examinations They found 90% accuracy in the use of urine cytology but it is important to note that they realized there should be screening programs to detect and study bladder pathology in the early stages to facilitate management and treatment

The eggs of different parasites have been detected incidentally in human vaginal and cervical smears [1] Urinary cytology has not been used as a technic to monitor the development of parasite infections in small animals although the passage of schistosome eggs in the excreta of rodents and nonhuman primates has served as a means of establishing patency or determining the efficacy of chemotherapy To our knowledge however cytology has not been employed previously to detect or follow the sequential development of pathology in the urogenital system of humans or laboratory animals

Much of the basic information on the schistosomiases including infection by *S haematobium* has been derived from studies employing common laboratory animals Observations regarding pathology of the bladder and parts of the urogenital system a feature of *S haematobium* infection in humans depends upon the use of nonhuman primates in which the disease more closely parallels that seen in humans The capuchin monkey–*S haematobium* combination has provided a means for multidisciplinary investigations with an emphasis on pathologic alterations of the urinary bladder Extensive experience with this model has led to the use of urinary cytology as a means of detecting and following in general disease processes and the evolution of urothelial alterations In the hands of an experienced investigator exfoliative cytology may be employed to judge certain basic host parasite relationships and to follow the course of bladder involvement and could be an important adjunct in evaluating the efficacy of drugs without killing the host

Acknowledgments

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References

1 Berry A Evidence of gynecologic bilharziasis in cytologic material A morphologic study for cytologists in particular Acta Cytol 15 487-498 1971

2 Berry A Multispecies schistosomal infections of the female genital tract detected in cytology smears Acta Cytol 20 361 365 1976

3 Cheever AW Kuntz RF Moore JA Bryan GT Brown RR Carcinoma of the urinary bladder in *Schistosoma haematobium* infection Animal model Proliferative urothelial lesions in nonhuman primates infected with *Schistosoma haematobium* Am J Pathol 84 673-676 1976

4 Cheever AW Kuntz RE Myers BJ Moore JA Huang TC Schistosomiasis haematobia in African hamadryas and gelada baboons Am J Trop Med Hyg 23 429-448 1974

5 Dimmette RM Sproat HF Klimt CR Examination of smears of urinary sediment for detection of neoplasms of bladder Survey of an Egyptian village infested with *Schistosoma haematobium* Am J Clin Pathol 25 103 1042 1955

6 El Bolkainy MN Ghoneim MA El Morsey BA Nasr SM Carcinoma of bilharzial bladder Diagnostic value of urine cytology Urology 3 319 323 1974

7 Elwi AM Fam Z Ramzy I Exfoliative cytology of the bilharzial ulcer and cancer of the urinary bladder J Egypt Med Assn 45 235 243 1962

8 Gill GW Exfoliative cytology Millipore Application Report AR 24 pp 21 23 1970

9 Kuntz RE Cheever AW Myers BJ Proliferative epithelial lesions of the urinary bladder of nonhuman primates infected with *Schistosoma haematobium* J Natl Cancer Inst 48 223 235 1972

10 Kuntz RE Moore JA Huang TC Distribution of egg deposits and gross lesions in nonhuman primates infected with *Schistosoma haematobium* (Iran) J Med Primatol 8 167 178 1979

11 Kuntz RE Moore JA Huang TC Schistosomiasis Use of nonhuman primates for understanding the human disease *In* Proceedings of the Seventh Congress of the International Primatological Society January 1979 Bangalore India

12 Kuntz RE Myers BJ Nonhuman primates for investigations of parasite oncology Proceedings of the Conference on Experimental Medicine and Surgery in Primates Lyon 1972 Part III Basel Karger 1972 pp 88 96

13 Myers BJ Kuntz RE Huang TC Moore JA Urinary bladder involvement in the talapoin (*Cercopithecus talapoin* Schreber) due to infection with *Schistosoma haematobium* (Bilharz 1852) Weinland 1858 Lab Animal Care 20 1004 1006 1970

14 Papanicolaou CN Atlas of Exfoliative Cytology Cambridge Massachusetts Harvard University Press 1963

15 Papanicolaou CN Marshall VF Urine sediment smears as a diagnostic procedure in cancers of the urinary tract Science 101 519 520 1945

A Simple Method for Study of the Same Cells by Light and Scanning Electron Microscopy

Ulrika V Mikel, C M I A C
Frank B Johnson, M D

A simple method of identifying cells for scanning electron microscopy (SEM) is described The method is very rapid and allows easy correlation between light microscopy and SEM Furthermore the procedure can be used with specially prepared cell suspensions as well as with routine smears

Several approaches have been proposed for using light and scanning electron microscopy (SEM) in the study of cells[1,2,4-6] *Our method requires no previously prepared microscopy slides with a target area where the cell preparation has to be placed*

Materials and Methods

An aliquot of cells from an ascitic fluid was centrifuged for ten minutes at 250 g The supernatant was discarded and the cells were washed once in Hanks balanced salt solution and once in 0 05 M sodium cacodylate buffer (pH 7 4) The cells were then fixed in 2% glutaraldehyde in cacodylate buffer at 4 C overnight After fixation the cells were washed for five minutes in cacodylate buffer the supernatant was discarded and the cells were resuspended in a small amount of buffer so that a concentrated cell suspension was obtained Cells from the suspension were dropped on slides coated with 0 1% polylysine[3] and stained by Gill s modified Papanicolaou method The slides were mounted with Permount (Fisher Scientific Company Pittsburgh Pennsylvania)

After mounting each slide was examined under the light microscope and pertinent and interesting areas were photographed The location of the field photographed was noted by recording the vernier readings of the graduated mechanical stage After photography the slide was dipped in xylene and the coverslip removed Two additional fresh changes of xylene were used to eliminate traces of the mounting medium and finally the slide was immersed in two changes of absolute ethanol for ten minutes The cell preparations were then critical point dried and coated with a thin layer of carbon followed by a thin layer of gold palladium

After the coating the slide was again placed under the light microscope The photographed areas were located according to the previously recorded mechanical stage readings Each area was enclosed in a circle of ink by using a marking device (Cell Finder Curtin Matheson Scientific Inc Washington DC) attached to the nosepiece An additional ink dot was placed at the top of the circle with a pen The slide was divided and cut into smaller pieces suitable for examination in a scanning electron microscope with each piece having one or several ink marks The slide pieces were then attached to support stubs and examined under a scanning electron microscope

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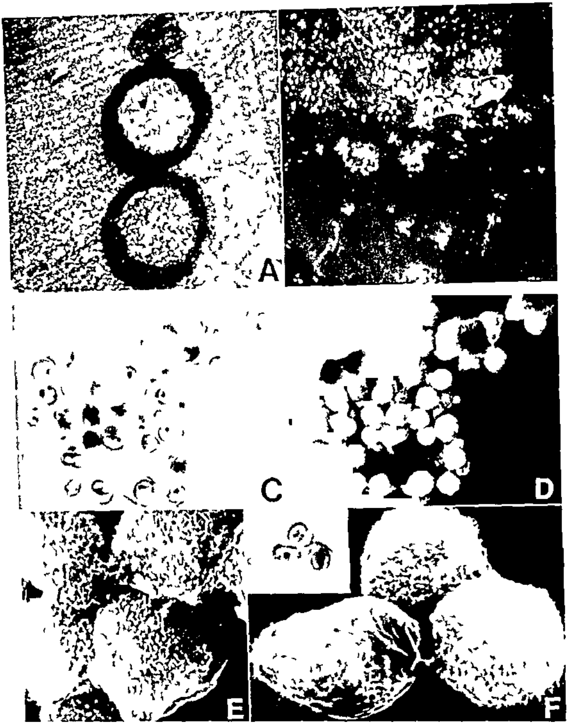

Figure 1
Cells from ascitic fluid from a patient with ovarian carcinoma (AFIP #79 12612) (A) The ink circles under low magnification in the scanning electron microscope (SEM) (× 24) (B) A partial ink circle with easily identifiable cell groups (× 70) (C and D) The cell group marked with the right arrow in Figure 1B as seen under both the light microscope and the SEM (× 475) (E) Detail of cells from the center of the cell group in Figure 1D (× 2 800) (F) High magnification of the small cell group (rotated 90) marked with the left arrow in Figure 1B (× 2 800) The inset shows the light photomicrograph of the same cell group

Results

Figure 1A shows an SEM photomicrograph with two ink circles The ink dot at the top of one circle helps in rotating the support stub so that the cells can be inspected in the same orientation as they were when seen under the light microscope This rotation can be accomplished from the outside of the scanning microscope A slightly higher magnification of a different ink circle depicts easily identifiable cell groups (Figure 1B) Higher magnification (Figures 1C and 1D) demonstrates that the cell group photographed by light microscopy (Figure 1C) can be easily identified as the same group when SEM is used (Figure 1D) More surface details are shown in Figures 1E and 1F

Discussion

The position of the light photomicrographed cells can be relocated after the cells are coated for SEM to within 1/10 of a millimeter on a good mechanical microscope stage A field of a little less than 1 mm in diameter can be observed with a 16× objective and even after coating the cells in question can be easily recognized They can therefore be positioned in the center of the viewing field before they are enclosed in the ink circle Under the scanning microscope it is then easy to find the ink marks The area inside the ink circles is small (1 mm in diameter) which in turn makes it easy to find the cells previously photographed by light microscopy

This method can make use of the entire area of a microscope slide The cells can be spread on the slide or dropped and allowed to settle on any part of the slide As long as care is taken to keep the cell preparation wet until the critical point drying routine smears can also be studied with this method for glutaraldehyde fixation is not essential Good results can be obtained with cells that have been fixed in 95% ethanol that is why this method lends itself so well to routine cytology and is also good for special studies

References

1 Bahr GF Bibbo M Mikel U Engler W Rao C Wied GL Cor
lation of light and scanning electron microscopy A new meth
for exfoliative cytology Acta Cytol 20 239 242 1976

2 Domagala W Kahan AV Koss LG A simple method of prep
ration and identification of cells for scanning electron micros
py Acta Cytol 23 140-146 1979

3 Mazia D Shatten G Sale W Adhesion of cells to surfac
coated with polylysine Applications to electron microscopy
Cell Biol 66 198-200 1975

4 Takenaga A Matsuda M Horai T Ikegami H Hattori S Sca
ning electron microscopy in the study of lung cancer New tec
nique of comparative studies on the same lung cancer cells b
light microscopy and scanning electron microscopy Acta Cyt
21 90-95 1977

5 Thornwaite JT Cayer ML Cameron BF Leif SB Leif RC
technique for combined light and scanning electron microscop
of cells IITRI/SEM 5 127 130 1976

6 Wetzel B Erickson BW Lewis WR The need for positive ident
fication of leukocytes examined by SEM IITRI/SEM 3 535-54
1973

Centrifugal Cytology of Nipple Aspirate Cells

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Robert DerHagopian M D
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Michael Silverman M D

Two improvements in the methodology for obtaining and preparing nipple aspirates from nonlactating women are reported The first is the development and use of a new breast pump with a controllable vacuum and cups of various sizes The second is the use of centrifugal cytology to prepare the dispersions Twenty one of 24 breasts of patients in the age range 30 to 49 years produced cellular dispersions which contained foam cells of them 13 contained ductal cells A comparison of glutaraldehyde and ethanol fixation indicated that the cells appeared substantially the same

Since the time of Papanicolaou[8,9] there have been attempts to determine if cytology of nipple aspirates of nonlactating women can be employed for early diagnosis of breast cancer Recently two groups performed extensive studies employing a Sartorius pump[11,12] to obtain the fluid subsequently preparing the specimen with an 8μm pore size Millipore filter (Millipore Corp Bedford Massachusetts) In a third study [1] of 1 744 women fluid aspirated with the Sartorius pump was smeared directly on a microscope slide The capacity to obtain fluid has been determined in an extensive study by Petrakis et al [10] These authors found that 78% of Caucasian 75% of black and 32% of Chinese women between the ages of 31 and 50 were secretors The low incidence of secretors among the Oriental populations presumably is because this population has a high proportion of individuals with dry type cerumen

Sartorius et al[11] have demonstrated that they can detect very early cancers (less than 0 8 cm in diameter) However lesions larger in diameter than 2 4 cm which are detectable by other means were not detectable by cytology presumably because the ducts were sealed off by these larger lesions The cytology of breast secretions of benign[3] and malignant[4] cells has recently been described Although these authors found that aspirate cytology had been of little value in diagnosing clinical cancer in this series of cases they did observe Cytologic abnormalities in the category of dysplasia/carcinoma *in situ* can be observed in a significant number of women who have malignant breast disease

If cytology of induced nipple aspirates is to serve as part of a cancer screening program the techniques for obtaining and preparing the specimens must maximize diagnostic accuracy while minimizing cost This paper reports on the combined use of centrifugal cytology[5] and a new breast pump which minimizes

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Results

Figure 1A shows an SEM photomicrograph with two ink circles The ink dot at the top of one circle helps in rotating the support stub so that the cells can be inspected in the same orientation as they were when seen under the light microscope This rotation can be accomplished from the outside of the scanning microscope A slightly higher magnification of a different ink circle depicts easily identifiable cell groups (Figure 1B) Higher magnification (Figures 1C and 1D) demonstrates that the cell group photographed by light microscopy (Figure 1C) can be easily identified as the same group when SEM is used (Figure 1D) More surface details are shown in Figures 1E and 1F

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1 Bahr GF Bibbo M Mikel U Engler W Rao C Wied GL Correlation of light and scanning electron microscopy A new method for exfoliative cytology Acta Cytol 20 239 242 1976

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3 Mazia D Shatten G Sale W Adhesion of cells to surfaces coated with polylysine Applications to electron microscopy J Cell Biol 66 198 200 1975

4 Takenaga A Matsuda M Horai T Ikegami H Hattori S Scanning electron microscopy in the study of lung cancer New technique of comparative studies on the same lung cancer cells by light microscopy and scanning electron microscopy Acta Cytol 21 90-95 1977

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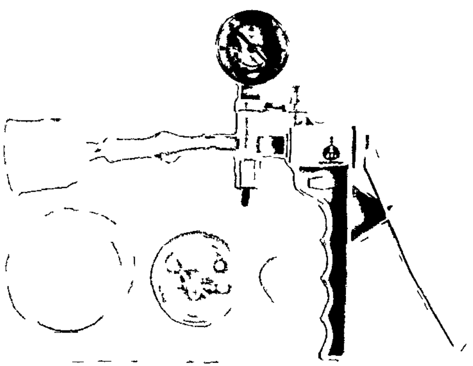

Figure 1
Breast pump A commercially available hand vacuum pump is attached via a short length of Tygon tubing to a female ground glass joint which holds the male joint of the three different-sized glass cups shown at the bottom of the figure At the top of the vertical column at the end of the pump is a vacuum gauge calibrated in inches and centimeters of mercury A pulldown tab at the bottom of this column releases the vacuum

the cost of the procedure and potentially maximizes the diagnostic accuracy

Materials and Methods

The new breast pump* (Figure 1) consists of a conventional hand vacuum pump vacuum gauge and interchangeable glass or plastic cups of varying sizes with smooth edges to fit over the nipple and areola The nipple and areola are cleaned with 0 9% sodium chloride saline solution and as much debris as possible is removed Cerumenex (Purdue Frederick Norwalk Connecticut) is then applied to the nipple and left in place for five minutes to dissolve encrusted areas Subsequently the nipple and areola are washed again and dried The suction apparatus is applied to the breast and negative pressures of 5 to 40 cm of mercury are achieved and maintained for one minute The use of interchangeable cups permits both compression of the breast and the application of negative pressure to the nipple When the pressure is released and the pump removed from the breast manual pressure is then applied in all breast quadrants until fluid is obtained (Figure 2) Moderate discomfort due to pressure pain was encountered but in no case did any of the volunteers request termination of the procedure No long term or persistent sequelae

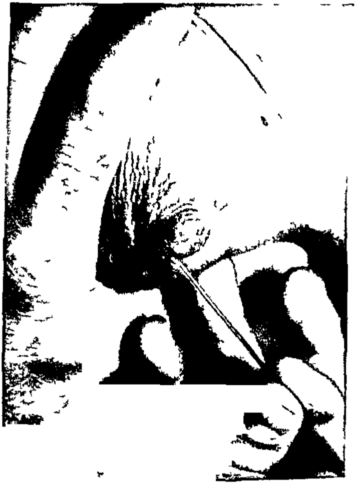

Figure 2
Compression of the right lower quadrant and removal of fluid with a capillary A small drop of fluid is shown at the edge and entrance of the capillary

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The Centrifugal Cytology Bucket is commercially available for *in vitro* diagnostic use from Newport Instruments PO Box 15435 Plantation FL 33318 which also supplies the special pump and cups for investigational use were encountered and several of our volunteers underwent several aspirations willingly

Most of our samples were obtained in 20µl capillaries (some required 80µl capillaries) which were emptied into 0 2 ml of saline or Mucolexx (Lerner Laboratories Stamford Connecticut) and washed out with more diluent the samples in saline were transported to the cytology laboratory at once and those in Mucolexx within 24 hours The use of Mucolexx permitted the samples to be prepared without interrupting the schedule of the cytology laboratory Each cell suspension was placed directly into a chamber of the 12 hole Centrifugal Cytology Bucket [7] Samples from the left and right breasts of the same subject were placed at opposite ends of the bucket Just recently a special transparent four hole ample block (Figure 3) has been developed for breast cytology The asymmetry of the layout of the sample chambers and the embossing of *L* and *R* at the ends should minimize the possibility of confusing the left and right breast samples The transparency of the block permits monitoring of the fluid level in the sample chambers

The buckets were centrifuged at 1 200 rpm (250 × g) for 10 minutes and then most of the samples fixed with 4% glutaraldehyde [8] The samples which had been transported in Mucolexx could also be fixed with 95% ethanol In either case the supernatant fluid was removed via the fluid outlet hole with a 1 ml syringe equipped with a 23-gauge needle and the glutaraldehyde or ethanol was subsequently added to the fixative cup After centrifugation at 1 200 rpm (250 × g) for ten minutes the Centrifugal Cytology Bucket was disassembled and the slide placed in 95% ethanol The slide was then stained according to the previously published procedure[8] except that for these nongynecologic preparations the hematoxylin solution consisted of 5 gm hematoxylin 100 gm ammonium alum 2 5 gm mercuric oxide 1 000 ml H_2O and 50 ml 95% ethanol For staining the solution is diluted with an equal volume of distilled water

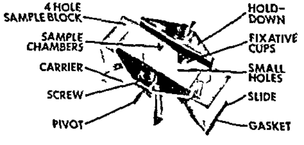

Figure 3
Four hole Centrigual Cytology Bucket developed for breast aspirates The bottom carrier sample chamber small holes and fixative cups are identical to those of the 1 hole Centrifugal Cytology Bucket *The trapezoidal layout and labelling of the sample chambers facilitates identification of the specimens*

Results

Preliminary results indicate that fluid is obtainable from breasts of women aged 20 to 73 To date 37 women have participated in this project Of 48 breasts pumped 41 yielded fluid of them 32 produced samples containing foam cells As shown in Table I if the data are broken down by age most of the nonproductive and unsatisfactory samples are found in the 20 to 29 year-old and 50 and over groups All of the women aged 30 through 49 produced fluid and of them 21 out of 24 preparations contained foam cells including those which also contained ductal cells Figure 4 shows a field which contains both foam and ductal cells

Of the 32 samples with foam cells 20 (62%) contained both ductal and foam cells and 12 (37 5%) foam cells only This finding is in agreement with that of King et al [3] who found ductal cells in 63 2% of the specimens obtained from nonpregnant women The calculated cellular composition except for ductal cells of each dispersion was determined from differential counts of three microscope fields and multiplication by 17 13 the ratio of the area of the dispersion to the sum of the areas of the fields Since the ductal cells were found as clumps it was necessary that the total number of ductal cells up to 100 be determined by scanning the entire dispersion The reproducibility of these differential counts was checked by count

Table I *Age Groups*

	20-29	30-39†	40-49‡	50 + §	Total
Women	17	1	4	4	37
Breasts	19	19	5	5	48
Unsatisfactory	5	2	1	1	9
No fluid	7	0	0	0	7
Samples containing foam cells	7	17	4	4	32
Foam cells only	1	7	1	3	12
Foam and ductal cells	6	10	3	1	20

All of the 20-29 group had apparently normal breasts
†In the 30-39 group one patient had fibrocystic disease two had [illegible] the other breast [illegible] done had a 2×1 5×1-cm [illegible] of breast which was sampled and produced both ductal and foam cells but [illegible] malignant [illegible]
‡In the 40-49 group there was one cystic breast and one with fibrocystic disease
§In the 50+ group there were [illegible] of the other breast and missed 3×2×2-cm can

Figure 4
Oil immersion (×25 lens) photomicrograph of foam cells and hyperplastic ductal cells in fibrocystic disease of the breast

ing two dispersions in triplicate The results given in Table II indicate that the standard deviation approximated the expected counting error which is the square root of the number of cells counted The results of the differential counts of ten dispersions of cells obtained from normal breasts from women between the ages of 30 and 49 are given in Table III The mean of the total recovery of cells in this study is approximately half that reported by King et al[3] However the large standard deviation in both studies indicates that the age distribution of the patients could significantly affect the results The median which is less sensitive to the few cases with large numbers of cells might be a more reliable statistic Table IV gives the results on similar differential counts of cells from five breasts with fibrocystic disease of women in the same 30 to 49 year-old age group The increased total cellularity of this group is due to the squamous and particularly the foam cells Figure 5 is a photomicrograph of the one papilloma which was found in this study

Figure 6 is a comparison of cells from a split sample where half were fixed in glutaraldehyde and the other half in ethanol For visual diagnostic purposes there appears to be no significant difference between the two preparations The alcohol fixation technique is slightly more rapid and the green cytoplasmic staining is presently lost with the glutaraldehyde fixation presumably because of a decrease in positive lysine residues[2] However the use of glutaraldehyde is advantageous in that it permits the reexamination of questionable samples by scanning or transmission electron microscopy[13]

Table II *Reproducibility of Cell Counts of Three Fields*

	RBC	WBC	Squamous	Foam	Ductal
Specimen #85					
	4	12	0	183	0
	5	11	0	165	0
	2	6	0	182	0
Average	3 7±1 5	9 7±3 2	0	177±10	0
Specimen #101					
	5	32	0	112	73
	3	22	0	121	75
	0	20	0	136	78
Average	2 7±2 5	25±6 4	0	123±12 1	75±2 5

All ductal cells were counted

Discussion

The previous smear or filter procedures for producing fixed stained dispersions of nipple and breast aspirates were time consuming and resulted in preparations which were more difficult to screen than those prepared by centrifugal cytology Since two of the chambers of the Centrifugal Cytology Bucket have been used successfully to prepare a fixed stained

Table III *Average Distribution of Cell Types from Normal Breasts*

	RBC	WBC	Squamous	Foam	Ductal	Total
Median	154	677	112	428	15	2 480
Mean	471	869	135	1 000	23	2 498
S D	886	1 003	173	977	26	1 794
Median %	7.1	39.6	4.6	31.2	1.0	
Mean %	17.9	35.8	7.9	37.0	1.3	100
S D of %	27.5	27.5	10.0	25.1	1.4	

A d al w o n d

Table IV *Average Distribution of Cell Types from Fibrocystic Breasts*

	RBC	WBC	Squamous	Foam	Ductal	Total
Median	0	206	959	2 95	19	4 780
Mean	45	740	846	19 477	27	20 963
S D	64	1 190	601	25 701	21	25 831
Median %	0	6.1	34.4	48.0	0.4	
Mean %	1.6	6.5	38.3	52.8	0.8	100
S D of %	2.3	6.7	41.3	43.2	0.9	

A d w d

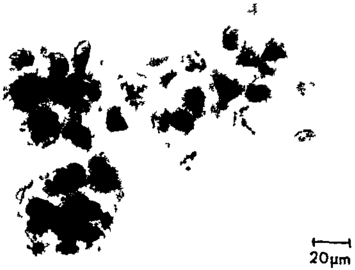

Figure 5
Oil immersion (×25 lens) photomicrograph of papillary aggregate of hyperplastic ductal cells in ductal papilloma of the breast

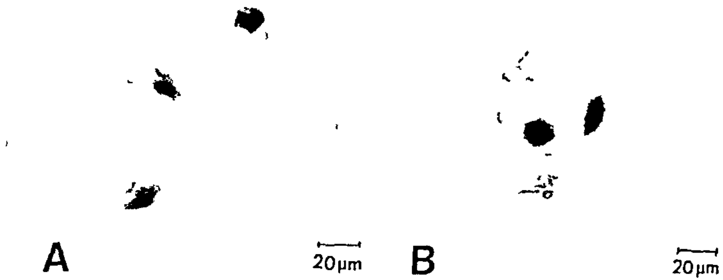

Figure 6

Foam cells of a breast with fibrocystic disease (A) fixed in alcohol and (B) fixed in glutaraldehyde. The quality of cellular details for both was comparable.

dispersion from the breasts of each patient (if the samples need dilution, four will suffice), it is now possible to rapidly prepare and conventionally screen samples containing all of the cells obtained from each breast on the same slide. This in itself should considerably increase the cost effectiveness of nipple secretion screening. In fact, the time it takes to screen one of these 8×8 mm squares is of the order of two minutes. Automated techniques for screening would not be cost effective unless useful information were obtained beyond that available from conventional screening.

Because of the small size of the data base, this paper has not established whether cytology will serve as a cost-effective component of breast cancer screening. It has been possible to diagnose fibrocystic disease and the associated increase in cellularity. However, in two cases we have verified the observation of Sartorius et al[12] that tumors detectable by other means can produce false negative samples. The results obtained with our new breast pump, where all sampled breasts of the women between ages 30 and 50 produced fluid, are clearly superior to those obtained in Buehring's extensive study with the Sartorius breast pump, where the percentage of fluid yielding women (one or both breasts) in this age group ranged from 50% to 67%. In terms of the entire population in our study, fluid was obtained from 85% of all breasts sampled and from one or both breasts of 80% of the patients (28/35). The latter percentage of secretors out of the total population may be compared with the results of Buehring, who obtained fluid from 846/1744 (49%) breasts overall and whose best results were 129/213 (60%) and with those of Petrakis et al, 109/152 (72%), excluding the low yielding Oriental population from his figures. This exclusion is necessary because the Miami area contains far fewer Orientals than does San Francisco. The use of a vacuum pump with a gauge will permit the physician to classify a woman objectively under standardized conditions of negative pressure and

Acknowledgments

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References

1 Beuhring GC Screening for breast atypias using exfoliative cytology Cancer 43 1778 99 1979

2 Hopwood D Theoretical and practical aspects of glutaraldehyde fixation *In* Fixation in Histochemistry Edited by PJ Stoward London Chapman and Hall 1973 pp 47 83

3 King EB Barrett D King M Petrakis NL Cellular composition of the nipple aspirate specimen of breast fluid I The benign cells Am J Clin Pathol 64 728 738 1975

4 King EB Barrett D Petrakis NL Cellular composition of the nipple aspirate specimen of breast fluid II Abnormal findings Am J Clin Pathol 64 739 748 1975

5 Leif RC Easter HN Jr Warters RL Thomas RA Dunlap LA Austin MF Centrifugal cytology I A quantitative technique for the preparation of glutaraldehyde-fixed cells for the light and scanning electron microscope J Histochem Cytochem 19 203 215 1971

6 Leif RC Gall S Dunlap LA Railey C Zucker RM Centrifugal cytology IV The preparation of fixed stained dispersions of gynecological cells Acta Cytol 19 159 168 1975

7 Leif RC Ingram D Clay C Bobbitt D Gaddis R Leif SB Nordqvist S Optimization of the binding of dissociated exfoliated cervicovaginal cells to glass microscopic slides J Histochem Cytochem 25 538 543 1977

8 Papanicolaou GN The value of exfoliative cytology in the diagnosis and control of neoplastic disease of the breast Ca 4 191 197 1954

9 Papanicolaou GN Holmquist DG Bader GM Falk EA Exfoliative cytology of the human mammary gland and its value in the diagnosis of cancer and other diseases of the breast Cancer 11 377-409 1958

10 Petrakis NL Mason L Ree R Sugimoto B Pawson S Catchpool F Association of race age menopausal status and cerumen type with breast fluid secretion in nonlactating women as determined by nipple aspiration J Natl Cancer Inst 54 892 933 1975

11 Sartorius OW Breast fluid cells help in early cancer detection JAMA 224 823 827 1973

12 Sartorius OW Smith HS Morris P Benedict D Friesen L Cytologic evaluation of breast fluid in the detection of breast disease J Natl Cancer Inst 59 1073 1080 1977

13 Thornthwaite JT Cayer ML Cameron BF Leif SB Leif RC A technique for combined light and scanning electron microscopy of cells *In* Scanning Electron Microscopy Part V IITRI/SEM/1976 pp 127 130

The Isolation and Identification of Exfoliated Prostate Cells from Human Semen

Margaret L Couture B S
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Cornelio P Katubig, Jr , M D

A prostatic acid phosphatase localization technique to identify exfoliated prostate cells in human semen is described Three morphologically distinct types of exfoliated prostate cells were observed in the semen of 14 vasectomized and nonvasectomized men (1) large ovoid prostate cells with abundant cytoplasm (2) small ovoid prostate cells with little cytoplasm and (3) columnar prostate cells These three types of exfoliated prostate cells were also observed within the lumina of prostatic alveoli when stained by this method Finally this staining procedure was found to have certain advantages over the conventionally used staining techniques for it allows the rapid identification of exfoliated prostate cells in human semen and provides a noninvasive technique for monitoring the functional state of the prostate gland

Artificial napthol phosphates in conjunction with diazonium salts were first used by Burstone to form insoluble azo dyes at the site of acid phosphatase activity in normal and malignant tissue sections [2, 7, 15] Goldberg and Barka also used this method to determine the acid phosphatase content of leukocytes [11] Since acid phosphatase is present in many tumors as well as in normal tissue it becomes critical in diagnosis to identify the organ from which the acid phosphatase originates [12] Of specific interest is the ability to distinguish between prostatic acid phosphatase and that from other organs Abu'l Fadl and Delory made this distinction possible when they discovered that acid phosphatase from the prostate is more resistant to inhibition by formalin than acid phosphatase from the liver erythrocytes kidney and spleen [1, 11] Using this information Carson compared frozen sections of malignant human tissue from the prostate lymph nodes and ovaries fixed in formalin and stained by the Burstone reaction method for acid phosphatase localization [5] In the tissue sections prepared in this manner she was able to identify and localize prostatic acid phosphatase Stonington working with malignant human prostatic epithelial cells in tissue culture also used formalin fixation in conjunction with the Burstone reaction method for acid phosphatase [17] He was able to isolate and distinguish prostatic epithelial cells from fibroblasts also growing in the culture by the former s ability to retain the formalin resistant prostatic acid phosphatase

This laboratory is the first to report the use of formalin fixation in conjunction with the Burstone reaction method in order to (1) identify exfoliated prostatic epithelial cells in the semen of healthy men (vasectomized and nonvasectomized) (2) compare the exfoliated prostate cells of the semen with prostate cells exfoliated into the lumina of prostatic alveoli and (3) compare the appearance of exfoliated prostate cells in semen as stained by the Papanicolaou method versus the Burstone method

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Materials and Methods

Triplicate semen specimens were obtained from each of ten nonvasectomized donors (25 to 40 years of age) and four vasectomized donors (30 to 50 years of age) Specimens from vasectomized men were used as controls since these specimens are devoid of germ cells (sperm precursors such as early spermatids) which are similar in size and appearance to prostate cells Each semen specimen was centrifuged at 900 g for 15 minutes and the cell pellet was resuspended in 10% neutral buffered formalin and incubated for 80 to 83 hours at 5 C Preliminary work indicated that 80 to 83 hours of incubation in formalin was optimal for leaching out or inactivating the nonprostatic acid phosphatase of the cellular elements present in semen The cells were then filtered through an 8μm Nuclepore polycarbonate filter to remove spermatozoa and white blood cells most of the exfoliated genitourinary tract cells were retained on the filter From each specimen two filters with attached cells were prepared and stained either by the Papanicolaou technique or by the Burstone reaction method described above

Prostate and seminal vesicle specimens were obtained from tissue removed by transurethral resection from three patients with benign prostatic hyperplasia and frozen sections were fixed in 10% neutral buffered formalin for five hours at 5 C after the method of Carson[8] The tissue required only a short period of incubation in the formalin solution since it did not contain as much acid phosphatase as did semen After incubation in formalin the tissue was stained by the Burstone reaction method and counter-stained with Harris hematoxylin

Results

Three morphologically distinct types of exfoliated prostate cells were observed in human semen (1) large round to ovoid prostate cells with abundant cytoplasm (15μm to 20μm in diameter) (2) small round to ovoid prostate cells with little cytoplasm (8μm to 10μm in diameter) and (3) columnar prostate cells (15μm to 20μm long and 8μm wide) These three types of prostate cells were also seen within the prostatic alveoli

Prostatic Tissue

Figure 1A shows a portion of the lumen of a prostatic alveolus and its surrounding basement membrane to which some basal cells (d) are attached A large prostatic cell (a) with a cellular diameter of 20μm and a nuclear diameter of 8μm may be seen within the lumen Pink-staining granules containing the formalin resistant prostatic acid phosphatase abound in the cytoplasm Also in the lumen both small (b) and columnar (c) prostate cells may be seen These two cell types may best be observed in Figure 1B where a small rounded prostate cell (b_1) with some nuclear detail and intensely staining acid phosphatase granules may be seen This cell is 10μm to 15μm in diameter and has a nuclear diameter of 8μm Several columnar prostate cells (c) with epicentrically placed nuclei and cytoplasmic acid phosphatase accumulations may be seen next to a large prostate cell (a) Figure 1C displays a group of five separate small prostate cells exhibiting little cytoplasm and a cellular diameter of 8μm to 10μm Figure 1D shows a grouping of five attached small prostate cells No nuclei are visible probably because their cytoplasm is completely filled with acid phosphatase granules Finally a columnar prostate cell with little acid phosphatase accumulation is depicted in Figure 1E These cell were observed within the lumen of the prostatic alveolus and all three types of prostate cells described above are similar in appearance to cells observed within prostatic tissue as illustrated by Reith and Ross[16] (Figure 3) Tweeddale[18] (Figure 9 2) and Franks[13] (Figures 18 and 20)

Semen

A large prostatic cell from the semen of a 50 year-old vasectomized man is seen in Figure 2A (compare with a in Figure 1A) This cell has a diameter of 15μm to 20μm and the cytoplasm is completely packed with acid phosphatase granules Two clear areas are seen in the cytoplasm The nucleus is 8μm in diameter and nucleoli are observed within the nucleus Figure 2B displays five attached small prostate cells from the semen of a 50 year-old vasectomized man which are similar to those seen in the lumen of the prostatic alveolus (Figure 1D) Their nuclei stained a light blue (arrow) and their cytoplasm is filled with pink-staining lysosomal granules Their cellular diameters are 10μm to 15μm and all have a nuclear diameter of 8μm A columnar prostate cell exhibiting few acid phosphatase granules may be seen in the semen of a 50 year-old vasectomized man (Figure 2C) and compared to the columnar prostate cell from tissue in Figure 1E Both cells exhibit both wide and narrow strips of cytoplasm on either side of a nucleus 8μm in diameter Both nuclei display some condensed chromatin

Figure 3A (compare with a in Figure 1A) depicts two large round exfoliated prostate cells occurring in the semen of a 25 year-old nonvasectomized

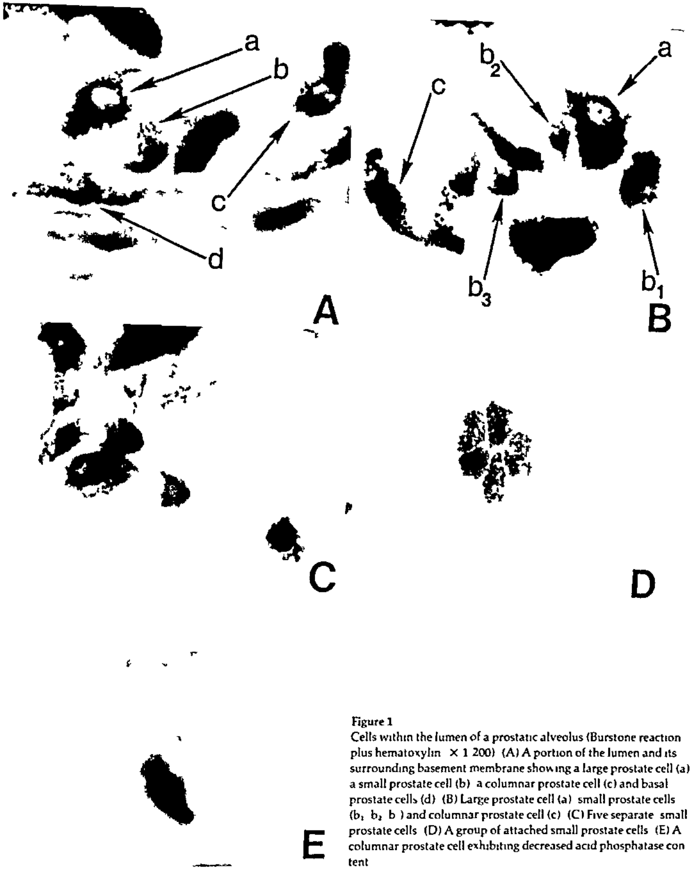

Figure 1
Cells within the lumen of a prostatic alveolus (Burstone reaction plus hematoxylin × 1 200) (A) A portion of the lumen and its surrounding basement membrane showing a large prostate cell (a) a small prostate cell (b) a columnar prostate cell (c) and basal prostate cells (d) (B) Large prostate cell (a) small prostate cells (b_1 b_2 b) and columnar prostate cell (c) (C) Five separate small prostate cells (D) A group of attached small prostate cells (E) A columnar prostate cell exhibiting decreased acid phosphatase content

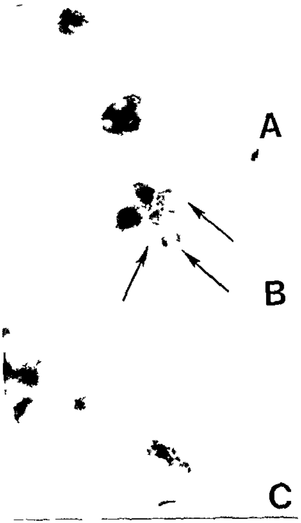

Figure 2
Exfoliated cells in the semen of a 50 year-old vasectomized male (Burstone reaction × 1 00) (A) Large prostate cell (B) Five attached small prostate cells the nuclei are pointed out by arrows (C) Columnar prostate cell

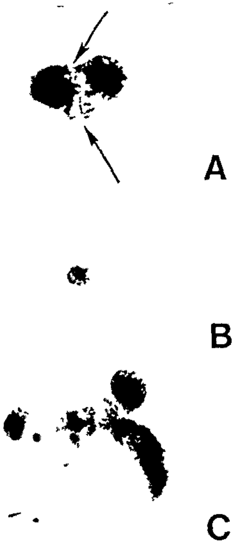

Figure 3
Exfoliated cells in the semen of a 5 year-old nonvasectomized male (Burstone reaction × 1 200) (A) Two large prostate cells their nuclei are pointed out by arrows (B) Small prostate cell (C) Columnar prostate cell

donor Each cell possesses an abundant amount of cytoplasm packed with acid phosphatase granules and an epicentrically placed nucleus (arrows)

Figure 3B (compare with Figure 1C) shows a small prostate cell seen in the specimen of a 25 year-old nonvasectomized man It has a cellular diameter of 8μm to 10μm and a nuclear diameter of 8μm It displays a small amount of cytoplasm containing a few acid phosphatase granules and a nucleus with visible chromatin patterns Figure 3C (compare with c in Figure 1B) displays a columnar prostate cell present in the semen of a 25 year-old nonvasectomized donor The cell exhibits a lightly stained nucleus of 8μm and its cytoplasm contains a large accumulation of acid phosphatase granules

Seminal Vesicle Tissue

Seminal vesicle tissue prepared by frozen section and stained for the presence of acid phosphatase may be seen in Figure 4 The individual epithelial cells seem to be slightly larger in cellular diameter than the prostatic epithelial cells The nuclei are round and

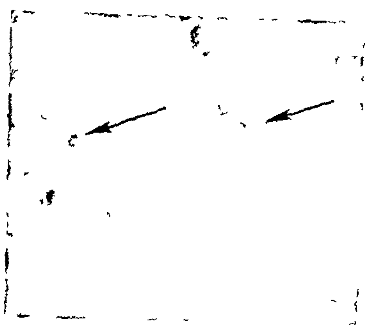

Figure 4

Seminal vesicle tissue Note the absence of acid phosphatase granules Arrow shows characteristic lipochrome inclusions (Burstone reaction plus hematoxylin × 1 200)

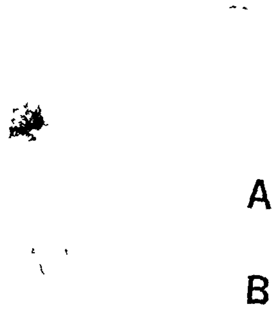

Figure 5

Papanicolaou-stained large exfoliated prostate cells in the semen of a 50-year-old vasectomized male (× 1 200) (A) Well-defined chromatin clumps in the nucleus and irregularly defined cytoplasm (B) Some nuclear detail visible

centrally placed and have chromatin flakes attached to the nuclear membranes The cytoplasm of exfoliated seminal vesicle cells does not contain acid phosphatase granules as seen in the epithelial cells of the prostatic tissue but does contain a distinctive lipochrome pigment (see illustration 4 9 in Tweeddale[18])

Papanicolaou Stain

Figure 5 (compare with Figure 1B and Figure 2A) shows two large exfoliated prostate cells occurring in the semen of a 50 year-old vasectomized man *The cells are stained by the Papanicolaou method Their cellular diameters range from 15μm to 20μm* and their nuclear diameters are 8μm to 10μm The cell in Figure *5A displays irregularly defined cytoplasm* and the nucleus contains well-defined chromatin clumps spread throughout the nucleoplasm Figure *5B shows a basal prostate cell Its cytoplasm is not* well defined but some nuclear detail is evident

Discussion

Using formalin fixation in conjunction with the Burstone reaction method three morphologically distinct types of exfoliated prostate cells were observed in the semen of vasectomized and nonvasectomized men (1) large round to ovoid prostate cells (2) small round to ovoid prostate cells and (3) columnar prostate cells Both the large cells (Figures 2A and 3A) and the small cells (Figures 2B and 3B) are similar in appearance to cells seen in prostatic massage secretions *as illustrated by Tweeddale* (Figures 9-4 and 9 6) [18] Both types of prostate cells probably represent the simple columnar (at times simple cuboidal *pseudostratified or squamous) prostatic epithelial* cells that have rounded up upon exfoliation The exfoliated columnar epithelial cells (Figure 3C shows cells similar to the elongated ones depicted in Figure 9 3 in Tweeddale[18]) have maintained their original shape perhaps due in part to their large accumulation of densely packed acid phosphatase granules Columnar cells containing very little acid phosphatase were also observed in the semen (Figure 2C) and prostatic alveoli (Figure 1E) of the older donors *These columnar cells are similar in appearance to the* fibromuscular stromal cells found in needle aspirates of prostate glands exhibiting benign prostatic hyperplasia as described and illustrated by Tweeddale (Figure 9-8) [18]

All three morphologic types were observed in both the semen and prostatic lumina The prostate cells seen in the lumina contain more acid phosphatase granules than their semen counterparts (compare Figures 1C 1D 2B and 3B) This is probably due to greater enzyme preservation achieved through frozen section preparation The seminal fluid prostate cells may also have released a portion of their acid phosphatase granules during the process of exfoliation and transit through the genitourinary tract The

columnar prostate cells exhibiting few acid phosphatase granules found in the semen of older men (Figures 1E and 2C) may represent a type of prostate cell which has ceased to maintain its normal secretory function due to the onset of benign prostatic hyperplasia as discussed by Tweeddale[18]

Figure 5 (compare with similar appearing cells obtained by prostatic massage in Tweeddale Figures 9 4 9 6 and 9 7) displays two exfoliated prostate cells present in the semen of a vasectomized donor These cells were stained by the Papanicolaou staining method and their identification would be quite difficult if other cells were also present in the semen specimen Human semen contains white blood cells immature and mature germ cells and four distinct populations of exfoliated genitourinary tract cells[9,10] (1) transitional cells of the bladder or of the membranous or prostatic urethra (2) squamous epithelial cells of the fossa navicularis (3) prostate cells and (4) seminal vesicle cells All of these cells are relatively easy to identify except for the prostate cells which look very much like lymphocytes and immature germ cells Thus the use of formalin fixation in conjunction with the Burstone reaction method for acid phosphatase permits the quick and positive identification of exfoliated prostate cells occurring in the semen

This laboratory is the first to report the use of this staining method to isolate and identify the exfoliated prostate cells present in human ejaculate[10] Furthermore these seminal fluid prostate cells seem to originate from prostate cells that are sloughed off or exfoliated into the lumina of the prostatic alveoli It is suggested that the staining method presented here could be used to effectively identify exfoliated prostate cells within the semen so that these cells could be quantitated and their morphology examined The diagnostic potential of this approach should be evaluated

Acknowledgments

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References

1 Abu'l Fadl MAM King EJ Properties of the acid phosphatases of erythrocytes and of the human prostate gland Biochem J 45 51 60 1949

2 Burstone MS Histochemical comparison of napthol AS phosphates for the demonstration of phosphatase J Natl Cancer Inst 20 601 609 1958

3 Burstone MS Histochemical comparison of napthol AS-substrates J Histochem Cytochem 6 87 88 1958

4 Burstone MS Histochemical demonstration of acid phosphatases with napthol AS phosphates J Natl Cancer Inst 21 523 532 1958

5 Burstone MS Histochemical demonstration of phosphatases in frozen sections with napthol AS phosphate J Histochem Cytochem 9 146 153 1961

6 Burstone MS The relationship between fixation and techniques for the histochemical localization of hydrolytic enzymes J Histochem Cytochem 6 322 339 1958

7 Burstone MS Keyes PH Studies on calcification I The effect of inhibition of enzyme activity on developing bone and dentin Am J Pathol 13 1229 1235 1957

8 Carson FL Acid phosphatase staining in the histopathology laboratory Am J Med Technol 39 333 337 1973

9 Couture ML Freund M A method for the isolation and identification of exfoliated prostatic cells in human semen *In* Proceedings of the Fourth Annual Meeting of the American Society of Andrology 19 9 p 19

10 Couture ML Freund M The presence of exfoliated genitourinary tract cells in human semen *In* Proceedings of the Third Annual Meeting of the American Society of Andrology 1978 p 16

11 Delory GE Sweetser TH White TA The use of formalin and alcohol in the estimation of prostatic phosphatase J Urol 66 724 733 1951

12 Dow SH Pretlow TG Separation of cells exhibiting acid phosphatase activity from disaggregated hamster prostate cells in an isokinetic gradient of Ficoll in tissue culture medium J Natl Cancer Inst 54 147 150 1975

13 Franks LM Benign prostatic hyperplasia Gross and microscopic anatomy *In* Benign Prostatic Hyperplasia Edited by JT Grayhack JD Wilson MJ Scherbenske Bethesda Maryland National Institutes of Health 1975 pp 63 89

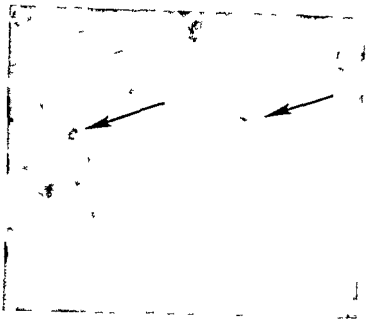
14 Goldberg AF Barka T Acid phosphatase activity in human blood cells Nature 195 297 1962

15 Kaplow LS Burstone MS Cytochemical demonstration of acid phosphatase in hematopoietic cells in health and in various hematological disorders using azo dye techniques J Histochem Cytochem 12 805 811 1964

16 Petch EJ Ross MH Atlas of Descriptive Histology Second edition New York Harper & Row 19 0 p 174

17 Stonington OG Szwec N Webber M Isolation and identification of the human malignant prostatic epithelial cells in pure monolayer culture J Urol 114 903 908 1975

18 Tweeddale DN Urinary Cytology Boston Little Brown & Co 1977 pp 31 139 144



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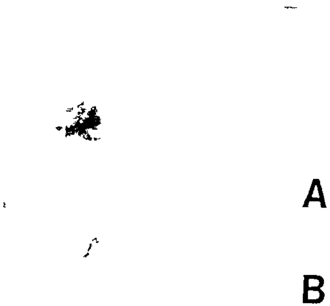

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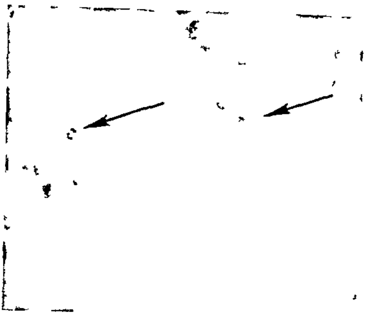

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Seminal vesicle tissue Note the absence of acid phosphatase granules Arrow shows characteristic lipochrome inclusions (Burstone reaction plus hematoxylin × 1 200)

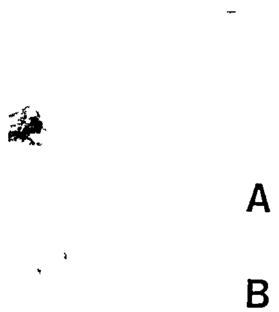

Figure 5

Papanicolaou-stained large exfoliated prostate cells in the semen of a 50 year-old vasectomized male (× 1 200) (A) Well-defined chromatin clumps in the nucleus and irregularly defined cytoplasm (B) Some nuclear detail visible

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This laboratory is the first to report the use of this staining method to isolate and identify the exfoliated prostate cells present in human ejaculate [9,10] Furthermore these seminal fluid prostate cells seem to originate from prostate cells that are sloughed off or exfoliated into the lumina of the prostatic alveoli It is suggested that the staining method presented here could be used to effectively identify exfoliated prostate cells within the semen so that these cells could be quantitated and their morphology examined The diagnostic potential of this approach should be evaluated

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Burstone MS Histochemical comparison of napthol AS phosphates for the demonstration of phosphatases J Natl Cancer Inst 20 601-609 1958

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11 Delory GE Sweetser TH White TA The use of formalin and alcohol in the estimation of prostatic phosphatase J Urol 66 724 733 1951

12 Dow SH Pretlow TG Separation of cells exhibiting acid phosphatase activity from disaggregated hamster prostate cells in an isokinetic gradient of Ficoll in tissue culture medium J Natl Cancer Inst 54 147 150 1975

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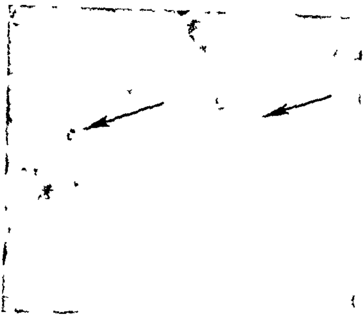
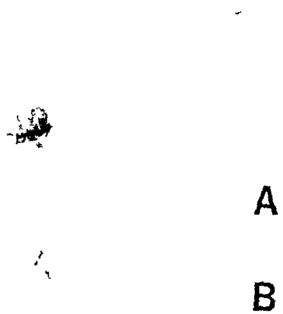


Figure 4
Seminal vesicle tissue Note the absence of acid phosphatase granules Arrow shows characteristic lipochrome inclusions (Burstone *reaction plus hematoxylin × 1 200)*



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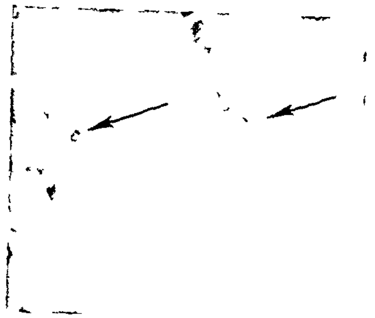

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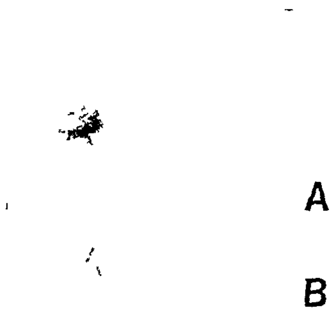

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intrauterine foreign body Mast cells or tissue basophilic granulocytes are well known to have receptors for IgE and their cytoplasmic granules contain histamine and heparin Our results suggest that mast cells may have an important role in the antifertility action or foreign body reaction of IUDs but further work is required to explain the mechanism of contraception

Acknowledgments

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References

1 Abrams RY Spritzer T Endometrial cytology in patients using intrauterine contraceptive devices Acta Cytol 10 240 245 1966

2 Bercovici B Gallily R The cytology of the retained IUD with relation to the mechanism of action Acta Cytol 22 456-459 1978

3 Gump DW Mead PB Horton EL Lamborn KR Forsyth BR Intrauterine contraceptive device and increased serum immunoglobulin levels Obstet Gynecol 41 259 264 1973

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5 Holub WR Reyner FC Forman GH Increased levels of serum immunoglobulins G and M in women using intrauterine contraceptive devices Am J Obstet Gynecol 110 362 365 1971

6 Parwaresch MR The Human Blood Basophil New York Springer Verlag 1976 pp 101 165

7 Sagiroglu N Phagocytosis of spermatozoa in the uterine cavity of women using intrauterine device Int J Fertil 16 1 14 1971

8 Sagiroglu N Sagiroglu E The cytology of intrauterine contraceptive devices Acta Cytol 14 58-64 1970

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Stellate Structures in Oral and Vaginal Smears

To the Editors

Stellate structures have been seen in oral and vaginal smears (Figure 1) in association with marked inflammatory changes of the cell population Cornelius[2] reported the same structures in skin scrapings of the normal and diseased skin The conditions in which they find habitat according to Cornelius included nummular dermatitis contact dermatitis tinea versicolor and manilial intertrigo The structures have stellate forms containing from three to eight legs joined at one point In Papanicolaou stain they attained a bleached yellow color revealing a thick walled structure and a hollow-space centrum which may be filled with a dark stain (hematoxylin) Cornelius suggested that the structures are of organic origin composed of cellulose The University of Pennsylvania School of Botany stated that these structures may be derived from the leaves and stems of *Viburnum dentatum* (arrowwood) which grows over much of the east coast and the midwest of the United States and Canada [1 3 7] In the *Encyclopedia of Plant Anatomy* Uphof[9] referred to these structures as trichomes

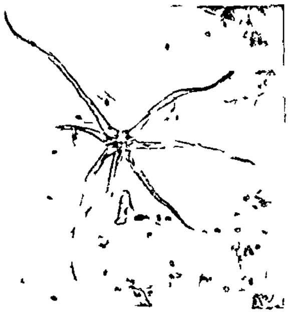

Figure 1
Typical stellate structure observed in routine cytologic smears (× 180)

Letters to the Editors

The Presence of Mast Cells in IUD Smears
A Preliminary Report

To the Editors

The cytologic study of the endometrial reaction to the intrauterine contraceptive device (IUD) has been discussed in a limited number of papers [1,2,8,10] Sağıroğlu et al[8] demonstrated the cellular changes in direct contact smears of the Lippes loop (loopal smears) Macrophages were found the first week after IUD insertion This was attributed to the antifertility effect of the IUD since spermatozoa were phagocytosed by macrophages [7] We confirmed the presence of macrophages surrounding the devices in six cases and other preparations showed polymorphonuclear leukocytes predominantly Previous authors did not mention the appearance of mast cells in loopal smears We have observed the presence of mast cells (tissue basophils) in direct-contact smears of IUDs (IUD smears) Mast cells were demonstrated with both Hansel s stain[4] and acidified toluidine blue (Figure 1) [6]

The total number of mast cells as observed with Hansel s method was counted microscopically Twenty Ota rings were removed as described by Sagıroglu [8] These were withdrawn within ten days after menstruation The devices had been in place one to seven years Touch preparations were made from the IUDs and from 20 controls without inflammatory changes

All patients including those in the control group were of reproductive age Large numbers of mast cells were observed in the presence of the IUDs but mast cells were infrequent in the control specimens (Table I) There was no correlation between the number of mast cells and the period the devices were retained in the uterine cavity On histologic study the IUDs were associated with a marked leukocytic infiltrate [9,11] Holub et al[5] and Gump et al[3] have reported elevated IgG and IgM levels in the serum of IUD users They postulated that these elevated immunoglobulins might reflect an allergic reaction to an

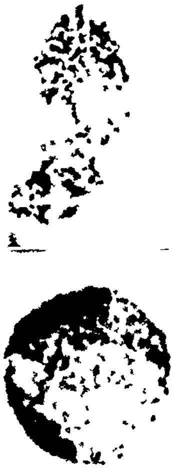

Figure 1
Different features of mast cells with large cytoplasmic granules in the IUD smears (Hansel s stain × 1 000)

Table I *Number of Mast Cells Per Two IUD Smears*

Group	Mean (±S D) number of mast cells per two slides
IUD smears (n = 20)	311 (±32)
Control smears (n = 20)	49 (±19)

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intrauterine foreign body Mast cells or tissue basophilic granulocytes are well known to have receptors for IgE and their cytoplasmic granules contain histamine and heparin Our results suggest that mast cells may have an important role in the antifertility action or foreign body reaction of IUDs but further work is required to explain the mechanism of contraception

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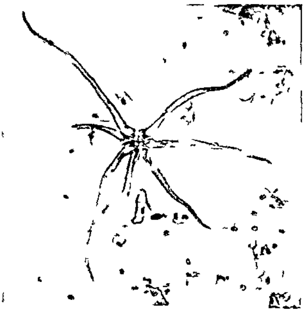

Figure 1
Typical stellate structure observed in routine cytologic smears (× 180)

Many species of plant may contain different types of trichomes varying greatly in color size shape and number[6,7] They may be arachnoid tufted T shaped or dendritic[2,6,8] Some of them like *Mucuna pruriens* contain the heat-stable chemical substance mucunain which is a proteolytic enzyme[8]

Trichomes present in the oral cavity and the vagina may contribute to the inflammatory changes and lysis of the cell populations seen in the smears

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References

1 Bailey LH Standard Cyclopedia of Horticulture Third volume London Macmillan & Co 1928 pp 3456-3464

2 Cornelius CE Shelley WB A unique stellate plant hair on human skin *Viburnum dentatum pubescence* Arch Dermatol 98 336 338 1968

3 Gleason HA The New Britton and Brown Illustrated Flora of the Northeastern United States and Canada Third volume Lancaster Pennsylvania Lancaster Press 1952 p 295

4 Laboratory *Procedures in Clinical Mycology* Air Force Manual 160-153 Washington DC US Government Printing Office 1963

5 Luniak B The Identification of Textile Fibers London Sir Isaac Pitman & Sons 1953 pp 3457 3464

6 McCrone WC Draftz RG Delly JG The Particle Atlas Ann Arbor Michigan Ann Arbor Science Publishers 1967 p 119

7 Metcalfe CR Chalk L Anatomy of Dicotyledons Second volume Oxford Claredon Press 1950 pp 750 754 756 758

8 Shelley WB Arthur RP Studies on cowhage (*Mucuna pruriens*) and its pruritogenic-proteinase mucunain Arch Dermatol 72 399-406 1955

9 Uphof JCT Hummel K Plants hairs *In* Encyclopedia of Plant Anatomy Fourth volume Berlin Nikolassee Gebruden Borntager 1962 p 5

Announcements

The International Academy of Cytology, Committee on Registration and Licensure
Successful Examinees

The following cytotechnologists have taken and successfully passed the Registry Examination for Cytotechnologists and are entitled to use the abbreviations C T (IAC) following their names

Tokyo Japan—August 16 1979

	Registration Number
Joshi Jinnouchi Notame 903 5 Minami ku Fukuoka shi Fukuoka ken 815 Japan	1785
Tomohiro Watanabe Suy 2659 Nishigo hi Kikuchi Sa hunso Kansha 16 Kum no 861 11 Japan	1786
Tai Sato Yamanodera 3 25 5 Izumi 981 31 Japan	1787
Itsuko Ikeda Nishigahara 43 14 1-chome Kita ku Tokyo 114 Japan	1788
Masami Sasaki Asashioka 2 3 Asashikawa Hokkaido 070 Japan	1789
Junko Kamak Tamagashi 1277-4 Okayama Okayama 701 21 Japan	1790
Keiko Kanda c/o Kiba Arai 4 24 5 Nakano ku Tokyo 165 Japan	1791
Yuko Yamamoto Nishitokuda 2 50 3 Yanaka-cho Shiba-gun Iwate 028 36 Japan	1792
Nikako Watanabe Wakabayashi so 2 go Arakawa 3-19 2 Arakawa ku Tokyo 116 Japan	1793
Toshimitu Shinohara Yakatabaru-danchi 17 3 Yakatabaru 33-1 Minami-ku Fukuoka Fukuoka 815 Japan	1794
Mitsuko Yoshikawa Aoyamab lu #72 Saginomiya 27 9 3 Naka ku Tokyo 165 Japan	1795
Emiko Ohtani Jujonakahara 4 10 26 Kita ku Tokyo 114 Japan	1796
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Many species of plant may contain different types of trichomes varying greatly in color size shape and number[6,7] They may be arachnoid tufted t-shaped or dendritic [2,6,8] Some of them like *Mucuna pruriens* contain the heat-stable chemical substance mucunain which is a proteolytic enzyme [8]

Trichomes present in the oral cavity and the vagina may contribute to the inflammatory changes and lysis of the cell populations seen in the smears

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References

1 Bailey LH Standard Cyclopedia of Horticulture Third volume London Macmillan & Co 1928 pp 3456-3464

2 Cornelius CE Shelley WB *A unique stellate plant hair on human skin Viburnum dentatum pubescence* Arch Dermatol 98 336-338 1968

3 Gleason HA The New Britton and Brown Illustrated Flora of the Northeastern United States and Canada Third volume Lancaster Pennsylvania Lancaster Press 1952 p 295

4 Laboratory Procedures in Clinical Mycology Air Force Manual 160 153 Washington DC US Government Printing Office 1963

5 Luniak B The Identification of Textile Fibers London Sir Isaac Pitman & Sons 1953 pp 3457 3464

6 McCrone WC Dratz RG Delly JG The Particle Atlas Ann Arbor Michigan Ann Arbor Science Publishers 1967 p 119

7 Metcalfe CR Chalk L Anatomy of Dicotyledons Second volume Oxford Claredon Press 1950 pp 750 754 756 758

8 Shelley WB Arthur RP Studies on cowhage (*Mucuna pruriens*) and its pruritogenic-proteinase mucunain Arch Dermatol 72 399-406 1955

9 Uphof JCT Hummel K Plants hairs *In* Encyclopedia of Plant Anatomy Fourth volume Berlin Nikolassee Gebruden Borntager 1962 p 5

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Ken Kubo [illegible] [illegible] Saitama [illegible] Japan	[illegible]
[illegible]	[illegible]
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[illegible]	[illegible]
[illegible]	[illegible]
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Russell S Haley 3401 Rancho Sierra Bakersfield California 93306	2017
Debra Judith Miller 26048 Farmfield Rd Calabasas California 91302	2018

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British Society for Clinical Cytology

The 18th Annual Meeting of the British Society for Clinical Cytology was held in Brighton from September 26 to 28 1979 This was a joint meeting with the Ninth European Congress of Cytology under the presidency of Arthur Spriggs The scientific program included the following symposia (1) the cell (2) serous fluids (3) menopausal hormone replacement therapy and (4) quality control in diagnostic cytology

There were numerous proffered papers on various aspects of clinical cytology technical advances cytogenetics etc and a slide seminar One session was reserved for the British Society of Colposcopy and Cervical Pathology under the chairmanship of Albert Singer The guest lecture delivered by Ralph Richart was The Diagnosis and Management of Cervical Intraepithelial Neoplasia and Vaginal Adenosis/Intraepithelial Neoplasia

Following the meeting workshops on aspiration cytology lymphomas viral diseases and urinary tract disorders were held at the Charing Cross Hospital London

At the AGM the officers of the society were reappointed for another year The resignation of the meeting secretary Dr David Melcher was received with regret and Dr Lever was elected to take his place Three council members were due to retire after three years of service and three new members (Dr Grubb Dr Yule and Mr Proctor) were elected

Greek Cytological Society

The officers of the Greek Cytological Society for the period January 1980 to December 1981 are the following

President	Kalliopi Alkalaï M D
Vice-President	Chryssi Frangaki M D
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The Sixth Annual Scientific Meeting of the Greek Medical Societies will be held May 8 to 11 at the Athens Hilton Hotel The Cytological Society will participate with a panel discussion entitled 'Bronchogenic Carcinoma and with eight papers on various cytologic subjects

Japanese Society of Clinical Cytology

The 21st Annual Scientific Meeting of the Japanese Society of Clinical Cytology will be held June 11–13 1980 in Chiba City Japan Professor Dr Hideo Katsuki Dean of Chiba University has been elected president of the congress The following items will be presented

1 Special lecture A Trial to Modulate Tumor Cell Differentiation (Dr Tokindo Okada)
2 Symposium on the diagnostic cytology of precancerous lesions (Chairmen Dr Ichiro Taki and Dr Yoshio Eui)
3 Symposium on the aspiration cytology of lymph nodes (Chairmen Dr Taizan Suchi and Dr Noboru Tanaka)
4 Lecture on the cell morphology of blood diseases (Dr Hideo Shigeta)
5 Slide seminar led by Dr Kinya Sawada and Dr Tatsuya Okamoto

For further information contact Dr Seigo Fukuma Director Chiba Cancer Center Hospital 666-2 Nitona Chiba 280 Japan

Sociedad Latino-Americana de Citologia

En ocasion del VI Congreso Latinoamericano de Citologia realizado conjuntamente con el V Congreso Argentino y II Reunion Iberoamericana de Citologia en la cuidad de Buenos Aires entre los dias 22 al 26 del mes de Octubre ultimo tuvo lugar la Asamblea Ordinaria de la Sociedad Latinoamericana de Citologia oportunidad en la cual fueron renovadas las autoridades de la Comision Directiva la que quedo constituida de la siguiente manera

Presidente	Dr Zenon Gonzalez Romero F I A C (Paraguay)
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Ademas deseamos llevar a su conocimiento que el VII Congreso Latinoamericano de Citologia tendra lugar en la cuidad de Asuncion Paraguay en el año 1982

ACTA CYTOLOGICA
THE JOURNAL OF CLINICAL CYTOLOGY

Editorial

Environmental Carcinogenesis and Cytology

Leopold G Koss M D F I A C

It has been known for several centuries that workers in cobalt mines in Schneeberg in Saxony Germany and in nearby radium mines in Joachimsthal (Jachymor) Czechoslovakia have had a high rate of lung cancer [3,9] The cause of the disease was shown to be radioactive dust that these workers inhaled This is one of the first and best known examples of environmental carcinogenesis The industrial revolution and more significantly perhaps the synthesis of organic chemical compounds in the 19th century increased the number of known carcinogens manyfold The first observations on the relationship of synthetic chemicals to cancer were recorded in 1895 when Rehn[10] described an excess of bladder cancer in workers in the dye industry producing aniline based colorants

The phenomenal industrial growth that occurred in the 20th century has polluted our air and water and has exposed the population of industrialized countries to contacts with many substances that have proven to be carcinogenic at least in the experimental animal [7] Ames s bacterial test can efficiently identify many mutagenic hence presumably carcinogenic substances [1] and their ubiquitous presence in urban air has been documented [2]

The dilemma that environmental carcinogenesis poses for humankind is very serious Clearly with a very few exceptions such as toxic war gases and the

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atom bomb it was not the intention of the engineers of industrial progress to harm people Indeed never before in the history of humankind have so many people lived as well and as long as they do today Sometimes unexpected benefits accrued to humankind even with the most devastating substances for example the most feared of all war gases mustard gas has become the cornerstone of chemotherapy for cancer and the technology initially used to produce an atomic explosion has been harnessed to produce energy that in spite of the Three Mile Island accident may serve us well until better solutions are found One of the terrible ironies of generalized economic progress and the increase in leisure time has been the widespread use of tobacco which contributed to a dramatic increase in lung cancer

It appears likely that virtually every synthetic chemical compound carries with it some risk to some people Industrial safety and reasonable measures to prevent massive exposure to potent carcinogens are clearly indicated However total elimination of all carcinogens from our environment does not appear likely A return to a simple rural society free of carcinogens would also mean a return to an economic setting wherein the inefficiency of the means of production would be such that it would take many hundreds of toiling workers to produce enough goods for a tiny elite In fact the developing countries aggressively seek industrial progress to raise the standard of living for their own populations disregarding for the time being at least the pollution and carcinogens that will inevitably follow

These comments may appear totally inappropriate in a journal devoted to clinical cytology were it not the fact that the study of cells has been used and ill continue to be used in many situations of known exposure to industrial and natural carcinogens Following the lead of Geoffrey Crabbe's work in England on urinary cytology in the search for bladder cancer in industrial workers at risk [3] other similar studies have been published in the United States and in Italy [4,8] The cytologic search for lung cancer in workers exposed to uranium has led to major studies on the natural history of this disease by Saccomanno and his associates [11] Similar work is in progress on workers exposed to asbestos in Tyler Texas [6]

Unfortunately there is no evidence to date that cytologic screening of industrial workers for either bladder or lung cancer has contributed significantly to the salvage of life In fact a British study flatly denied that there were any significant benefits of cytologic screening for bladder cancer [5]

Perhaps the most frustrating aspect of environmental carcinogenesis is the fact that only a relatively small proportion of people exposed to potent carcinogens develop cancer within their lifetimes [7] While the cleaning of our environment is a noble goal the identification of people at risk may prove to be a simpler cheaper and perhaps more efficient approach

There are very few clues known to us today to begin a successful search for the individuals at risk Is it the way in which they metabolize the carcinogens? Is it a deficiency or excess of some enzyme or group of enzymes? Is it an abnormal response of the target organ? Or is it a combination of these and yet other factors? Saccomanno emphasized squamous metaplasia of the respiratory epithelium as the first step in carcinogenesis in the bronchus [11] We can identify cell changes that place women at risk for carcinoma of the cervix This modest beginning is clearly worthy of further study The answer may well lie in cytology

References

1 Ames BN Durston WE Yamasaki E Lee FD Carcinogens are mutagens A simple test system combining liver homogenates for activation and bacteria for detection Proc Natl Acad Sci USA 70 2281 2285 1973

2 Commoner B Madyastha P Bronsdon A Vithayathil AJ Environmental mutagens in urban air particulates J Toxic Environ Health 4 59 77 1978

3 Crabbe JGS Exfoliative cytologic control in occupational cancer of the bladder Br Med J 2 1072 1076 1952

4 Forni A Ghetti G Armelli G Urinary cytology in workers exposed to carcinogenic aromatic amines A six year study Acta Cytol 16 142 145 1972

5 Fox AJ White GC Bladder cancer in rubber workers Do screening and doctor's awareness distort the statistics? Lancet 1 1009 1011 1976

6 Greenberg SD Hurst GA Matlage WT Miller JM Hurst IJ Mabry LC Tyler asbestos workers program Ann NY Acad Sci 271 353 364 1976

7 Hueper WC Occupational Tumors Springfield Illinois Charles C Thomas 1942

8 Koss LG Melamed MR Kelly RE Further cytologic and histologic studies of bladder lesions in workers exposed to para aminodiphenyl Progress report J Natl Cancer Inst 43 233 243 1969

9 Pirchan A Sikl H Cancer of the lung in the miners of Jachymor Am J Cancer 16 681 722 1932

10 Rehn L Blasengeschwulste bei Fuchsin-arbeitern Arch Klin Chir 50 588-600 1895

11 Saccomanno G Archer VE Auerbach O Saunders RP Brennan LM Development of carcinoma of the lung as reflected in exfoliated cells Cancer 33 256-270 1974

Expanded Cytologic Criteria for the Diagnosis of Adenocarcinoma in Situ *of the Cervix and Related Lesions*

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Fifty two cases of adenocarcinoma in situ *(AIS) of the cervix uteri or lesions closely related to AIS have been reported by this laboratory over a five year period Whereas this lesion has commonly been considered a rare and usually incidental histologic finding the large majority of these cases involved glandular atypia only and were prospectively diagnosed*

Comparison of the findings from this larger series with the 11 cases of AIS described by us previously[4] *has led to a shift in emphasis from cell size to sheet architecture as the primary cytologic criterion for diagnosis of this lesion The structure of AIS sheets was consistent with respect to the cytologic appearances described here even when the cells were poorly preserved*

Retrospective studies of Papanicolaou smears of other cases from this laboratory that were confirmed histologically as endocervical dysplasia microinvasive or early invasive adenocarcinoma or deeply invasive adenocarcinoma indicate that these entities can be distinguished from AIS primarily on the basis of the architecture of the sheets of cells Cellular groups comprising small crowded nuclei were a feature of several undercalled deeply invasive adenocarcinomas and the diagnostic significance of this material is discussed

Adenocarcinoma *in situ* (AIS) has been diagnosed in this laboratory more commonly than would have been expected from reports from other sources where it was typically a rare or incidental observation[4] Fifty two cases involving AIS or a lesion closely related to it have now been reported by us over a five year period and in only six of these was the glandular atypia an incidental finding The 52 histologic confirmations comprised 19 cases of AIS 3 cases of endocervical dysplasia 19 cases of microinvasive or early invasive adenocarcinoma and 11 cases of deeply invasive adenocarcinoma On the basis of this larger series we now present a refinement of the diagnostic criteria of AIS outlined previously by us in addition to a description of the cytologic presentation of endocervical dysplasia microinvasive or early invasive adenocarcinoma and deeply invasive adenocarcinoma All cytology for each case was critically reviewed in relation to the accuracy of the cytologic diagnosis within each of the four histologic groups

Materials and Methods

The 52 cases reported here were accumulated over a five year period in the course of a screening program involving approximately 250 000 Papanicolaou smears per annum Histologic confirmation was obtained for each case and in most cases the sections were reviewed by us Representative histologic slides were retained together with the current smears of each case and with any other smears received from the patient since 1962

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Results

The cases studied were divided into four groups according to the histologic diagnosis (Table I). The mean age of 19 patients with AIS was 39 years and the age range 22 to 66 years; for three patients with endocervical dysplasia the mean age was 36 years and the age range 33 to 38 years. Nineteen cases of microinvasive adenocarcinoma showed a mean age of 44 years and an age range of 29 to 57 years, and in 11 cases of deeply invasive adenocarcinoma the mean age was 49 years and the age range 34 to 63 years. Smears from all cases of endocervical dysplasia and AIS were taken routinely, and the cervix appeared benign or eroded. Abnormal vaginal bleeding prompted the smear in 10.5% of microinvasive cases and in 10.5% of cases the cervix was clinically suspicious. In 27.2% of the deeply invasive adenocarcinomas P.V. bleeding was noted, and in 54.5% the cervix was clinically suspicious.

All of the 52 patients represented in Table I were parous, with the exception of one patient with AIS and another with microinvasive adenocarcinoma. Hormonal contraception had never been administered to 25.3% of the AIS cases, 21.0% of the microinvasive cases and 33.3% of those with deeply invasive adenocarcinoma.

Group I: Adenocarcinoma in Situ

Diagnostic Accuracy. Adenocarcinoma in situ was prospectively diagnosed in 12 cases of pure AIS and 3 cases also showing squamous atypia (Table II). Smears previously reported as negative in five of these cases showed either endocervical dysplasia or AIS. Microinvasion was diagnosed in one case of poorly differentiated AIS, and invasion was predicted in one case of pure AIS.

Exfoliation Pattern. Sheets of cells and tissue fragments in the form of cellular strips and rosettes provided the diagnostic criteria of AIS. There was wide variation in the amount of abnormal material seen in individual smears, but in none of the 19 cases studied were single cells of diagnostic value.

Due to the crowding and overlapping of nuclei, sheets of AIS very rarely retained the honeycomb formation of normal endocervical material. The brush border of endocervical cells was also often

Table I *Summary of Clinical Data from 52 Cases Involving AIS or Related Lesions*

	Group			
	1 AIS	2 Endocervical dysplasia	3 Microinvasive or early invasive adenocarcinoma	4 Deeply invasive adenocarcinoma
Total no. of cases	19	3	19	11
Age				
Mean	39	36	44	49
Range	22-66	33-38	29-57	34-63
Reason for smear				
Routine	18	3	17	8
Abnormal bleeding	—	—	2	3
Not stated	1	—	—	—
Clinical appearance of cervix				
Normal	12	2	9	3
Eroded	7	1	8	2
Suspicious	—	—	2	6
Parity				
0	1	—	1	—
1-2	8	3	6	6
3-4	9	—	6	2
5+	1	—	4	2
Not stated	—	—	2	1
Hormonal contraception used				
Never	5	—	4	4
Previously	5	1	[illegible]	4
Currently	8	2	[illegible]	2
Not stated	1	—	[illegible]	

obliterated along the margins of sheets rendering their identification more difficult Openings within crowded sheets occasionally retained a clearly columnar structure and good evidence of the glandular nature of otherwise problematic sheets could be obtained from them

Where definitive gland openings could not be found and cytoplasm had been lost from sheet edges the appearance of pseudostratification of nuclei similar to that seen in histologic sections of AIS was of paramount importance Pseudostratified oval nuclei exhibited a typically uniform polarity with their *long axes at right angles to the luminal margin in* sheets of AIS Nuclei stripped of cytoplasm at the periphery of sheets retained this polarity but formed an irregular edge due to the varying levels at which nuclei were positioned within the pseudostratified epithelium This gave a frayed or feathered appearance to the edges of sheets (Figure 1) It was indeed possible to identify as AIS sheets with an otherwise indeterminate crowded appearance by observing quite small foci of feathered edging

The cytologic presentation of smaller tissue fragments correlated even more closely with the histologic appearance of AIS than was the case with larger sheets and these fragments usually provided the key to an accurate diagnosis Small strips of cells were particularly informative and often retained an intact brush border with crowded nuclei of uniform polarity placed at different levels throughout the thickness of the epithelium Small circular groups of cells or rosettes with peripheral nuclei and cytoplasm directed toward the center occurred in AIS as well as in endocervical atypicality However they were useful in the definitive diagnosis of AIS only when their nuclei exhibited crowding and pseudostratification Well preserved rosettes and strips of cells are illustrated elsewhere[4]

Nuclear Size The poor definition of individual cell outlines due to crowding effects rendered changes in the nuclear-cytoplasmic (N/C) ratio of AIS material difficult to quantify An essentially normal N/C ratio was evident however wherever small groups of cells were suitably spread and on this basis relative changes in nuclear size have been taken in this study to reflect changes in cell size

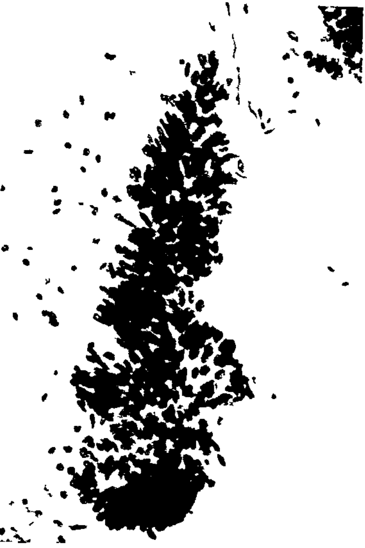
Nuclear size within sheets of AIS was typically uniform Sheets of AIS often contained larger nuclei than their benign counterparts but not exclusively

Table II *Prospective Cytologic Diagnoses and Histologic Confirmations for 19 Cases of AIS (Group I)*

Case no	Histologic confirmation	Date of diagnosis	False negative smears (year and grade)
Prospective diagnosis pure AIS			
1 (PD)	Pure AIS	12/73	1972 (*AIS*)
3 (JG)	Pure AIS	9/74	
4 (ED)	Pure AIS	10/74	1972 (endocervical dysplasia)
5 (PM)	Pure AIS	3/75	
6 (EM)	Pure AIS	4/75	
7 (CL)	Pure AIS	9/75	1974 (endocervical dysplasia)
9 (CR)	Pure AIS	5/76	
10 (SB)	Pure AIS	8/76	
13 (JF)	Pure AIS	5/76	
15 (NM)	Pure AIS	11/76	1969 (endocervical dysplasia) 1971 74 (AIS)
48 (KH)	Pure AIS	6/ 8	
Prospective diagnosis AIS			
2 (GV)	AIS poorly differentiated	6/74	
20 (KW)	AIS poorly differentiated	4/78	
8 (MS)	AIS + CIS	9/75	1971 72 (AIS)
14† (PS)	AIS + severe squamous dysplasia	8/76	
19† (ML)	AIS + CIS	11/76	
Prospective diagnosis adenocarcinoma of cervix + squamous dysplasia			
17 (CC)	AIS + severe squamous dysplasia	3/77	
Retrospective diagnosis			
11 (GG)	AIS + CIS	9/73	
12 (TD)	AIS + CIS	9/75	

Possibly mi [illegible]

†AIS + CIS



gure 1
ase 1 Feathered edges on a sheet of adenocarcinoma *in situ* aterial Nuclei denuded of columnar cytoplasm retain their po rity and pseudostratification (Papanicolaou-stained cervical rape × 180)

Figure 2
Case 45 Sheets of adenocarcinoma *in situ* showing nuclear size closely similar to that of adjacent intermediate squamous cells Note the feathered sheets at 1 o clock and 9 o clock and the sheet in which pseudostratified nuclei enclose a gland opening at 4 o clock Two small pseudostratified strips of columnar cells lie together at 7 o clock (Papanicolaou-stained cervical scrape × 110)

Variations in nuclear size from sheet to sheet oc irred within smears and this variation not infre- iently included AIS material with nuclei that were it larger than those of normal endocervical cells In ie case the AIS material predominantly comprised clei no larger than those of normal endocervical lls (Figure 2) Sheets of AIS-containing nuclei no ger than those of benign endocervical cells were en found to occur in the numerous previous false- gative smears reviewed during this study In sever other cases AIS material was seen in the absence normal endocervical cells Adenocarcinoma *in situ* is recognized in these smears on the basis of the oliation patterns rather than on the basis of in- ased nuclear size relative to that of normal endo vical cells

Nuclear Shape The chromatinic membrane was smooth and evenly stained and variation in nuclear shape was minimal The nucleus was round or more commonly oval when viewed laterally

Chromatin Pattern Chromatin was evenly dispersed throughout the nucleus A range from fine to coarse uniform granulation occurred with moderate granulation the most common finding Chromasia was variable and ranged from mild to marked hyperchromasia However in well preserved cells free of bloodstaining or inflammatory effects chromasia was clearly increased as compared with normal cells

Nucleoli Nucleoli were rare and small in all but 3 of the 19 cases studied These three cases are mentioned below in relation to inflammatory changes in AIS

Mitotic Figures Occasional mitotic figures were seen in each case

AIS in Inflammatory Smears The diagnostic smears from 11 of the 19 cases of AIS showed marked inflammatory changes The diagnosis was nevertheless correctly made in all cases but one where invasion was predicted on the basis of very degenerate cell groupings indicative of poorly differentiated adenocarcinoma In the other ten cases the salient features of the exfoliation pattern of AIS were retained Pseudostratification was clearly evident in sheets small strips and tissue fragments (Figures 3 and 4) Where the edges of sheets were devoid of cytoplasm evidence of pseudostratification was seen in the feathering effect noted previously

The relationship between AIS nuclei and therefore the overall architecture of AIS smears was retained even in those sheets where nuclear degeneration was evident Early degenerative changes such as nuclear ballooning or shrinking posed no real diagnostic problem since they were more often of a uniform nature within groups of cells and since cell size per se was not seen as an essential criterion of AIS Pseudostratification was retained in fact in material that had degenerated to the point that variations in the size and shape of adjacent nuclei were apparent and the structure of nuclear chromatin was lost Size variations of this magnitude were not typical of AIS and this material was too degenerate for diagnostic purposes The architecture of AIS was nevertheless

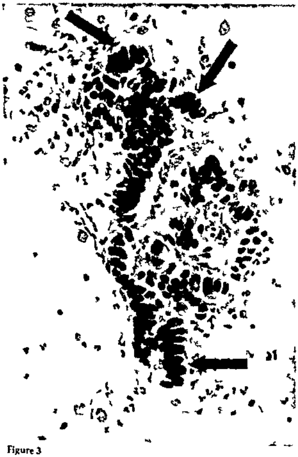


Figure 3
Case 13 Strips of cells (arrowed) showing the polarized nuclear crowding and pseudostratification typical of adenocarcinoma *in situ* in a heavily inflamed cervical scrape (Papanicolaou stain × 180)

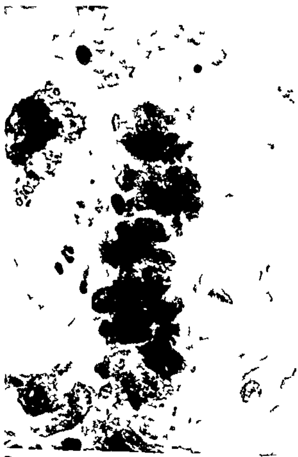


Figure 4
Case 19 Residual pseudostratification in a strip of adenocarcinoma *in situ* cells showing degenerative nuclear changes (Papanicolaou stain × 450)

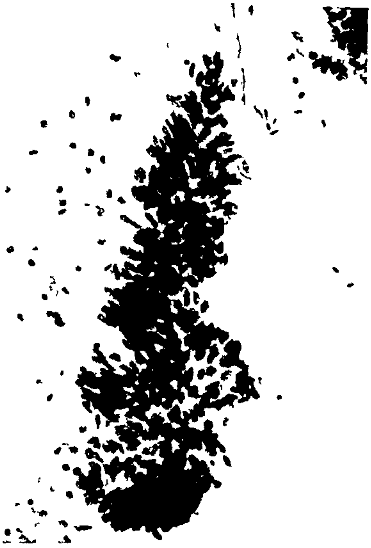


Figure 1

Case 1 Feathered edges on a sheet of adenocarcinoma *in situ* material Nuclei denuded of columnar cytoplasm retain their polarity and pseudostratification (Papanicolaou-stained cervical scrape × 180)

Figure 2

Case 48 Sheets of adenocarcinoma *in situ* showing nuclear size closely similar to that of adjacent intermediate squamous cells Note the feathered sheets at 1 o clock and 9 o clock and the sheet in which pseudostratified nuclei enclose a gland opening at 4 o clock Two small pseudostratified strips of columnar cells lie together at 7 o clock (Papanicolaou-stained cervical scrape × 110)

so Variations in nuclear size from sheet to sheet occurred within smears and this variation not infrequently included AIS material with nuclei that were not larger than those of normal endocervical cells In one case the AIS material predominantly comprised nuclei no larger than those of normal endocervical cells (Figure 2) Sheets of AIS-containing nuclei no larger than those of benign endocervical cells were often found to occur in the numerous previous false negative smears reviewed during this study In several other cases AIS material was seen in the absence of normal endocervical cells Adenocarcinoma *in situ* was recognized in these smears on the basis of the exfoliation patterns rather than on the basis of increased nuclear size relative to that of normal endocervical cells

Nuclear Shape The chromatinic membrane was smooth and evenly stained and variation in nuclear shape was minimal The nucleus was round or more commonly oval when viewed laterally

Chromatin Pattern Chromatin was evenly dispersed throughout the nucleus A range from fine to coarse uniform granulation occurred with moderate granulation the most common finding Chromasia was variable and ranged from mild to marked hyperchromasia However in well preserved cells free of bloodstaining or inflammatory effects chromasia was clearly increased as compared with normal cells

Nucleoli Nucleoli were rare and small in all but 3 of the 19 cases studied These three cases are mentioned below in relation to inflammatory changes in AIS

Mitotic Figures Occasional mitotic figures were seen in each case

AIS in Inflammatory Smears The diagnostic smears from 11 of the 19 cases of AIS showed marked inflammatory changes The diagnosis was nevertheless correctly made in all cases but one where invasion was predicted on the basis of very degenerate cell groupings indicative of poorly differentiated adenocarcinoma In the other ten cases the salient features of the exfoliation pattern of AIS were retained Pseudostratification was clearly evident in sheets small strips and tissue fragments (Figures 3 and 4) Where the edges of sheets were devoid of cytoplasm evidence of pseudostratification was seen in the feathering effect noted previously

The relationship between AIS nuclei and therefore the overall architecture of AIS smears was retained even in those sheets where nuclear degeneration was evident Early degenerative change such as nuclear ballooning or shrinking posed no real diagnostic problem since they were more often of a uniform nature within groups of cells and since cell size per se was not seen as an essential criterion of AIS Pseudostratification was retained in fact in material that had degenerated to the point that variations in the size and shape of adjacent nuclei were apparent and the structure of nuclear chromatin was lost Size variations of this magnitude were not typical of AIS and this material was too degenerate for diagnostic purpose The architecture of AIS was nevertheless

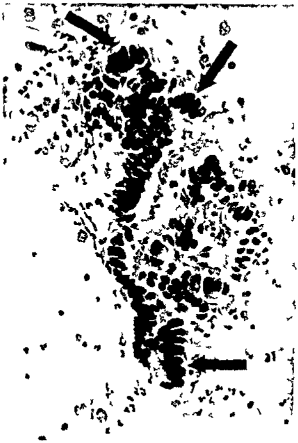


Figure 3
Case 13 Strips of cells (arrowed) showing the polarized nuclear crowding and pseudostratification typical of adenocarcinoma *in situ* in a heavily inflamed cervical scrape (Papanicolaou stain X 180)

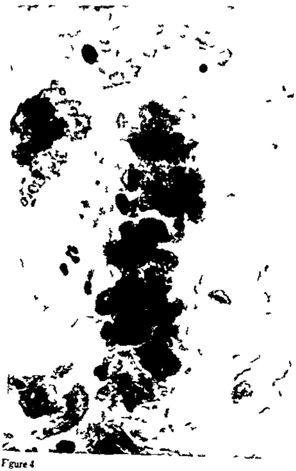


Figure 4
Case 19 Residual pseudostratification in a strip of adenocarcinoma *in situ* cells showing degenerative nuclear changes (Papanicolaou stain X 4[illegible])

retained, and on this basis such material should alert the observer to the possibility of glandular atypicality, particularly since such structures were rarely, if ever, seen as a product of degeneration in normal endocervical cells.

Nucleoli were equally rare and inconspicuous in the AIS material of inflammatory smears, as was the case for noninflammatory smears. In one case with markedly inflammatory smears, nucleoli were prominent in cell groupings suggestive of endocervical dysplasia but were relatively inconspicuous in the AIS material. Two other cases of AIS showing prominent nucleoli were seen histologically as poorly differentiated variants. Similar AIS material in which prominent nucleoli were present was seen in the previous false negative smear of a deeply invasive adenosquamous carcinoma (case 33, group 4, below).

Group 2: Endocervical Dysplasia

In this study, endocervical dysplasia is defined as a lesion showing a combination of architectural abnormalities of the columnar epithelium of the uterine cervix similar to, but less marked than, those described in AIS.

It should be noted that nuclear irregularities often exceeding those of AIS are seen in endocervical dysplasia, and histologically more heterogeneity can occur due to the presence of more differentiated mucin-containing cells in the epithelium.

Diagnostic Accuracy: One of the three cases of histologically confirmed endocervical dysplasia was prospectively diagnosed from smears. Microinvasive adenocarcinoma and invasive adenocarcinoma were the two other predictions (Table III).

Exfoliation Pattern: Crowded sheets of endocervical cells, columnar strips and rosettes were commonly seen in the two smears that yielded the correct prediction of endocervical dysplasia (Figures 5 and 6). However, dense crowding and marked pseudostratification of nuclei were rarely seen. Vertical displacement of nuclei prevailed in strips of cells viewed laterally, but this was less pronounced than that seen in pseudostratified AIS material. Adjacent nuclei in these strips were not crowded and laterally compressed as in AIS, and their polarity was less well developed.

Nuclear Size: Nuclear dimensions were more variable than in AIS. In most of the larger and more closely crowded sheets, nuclei were of uniform size and only slightly enlarged (Figure 5). Obvious nuclear enlargement was not uncommon, however, in smaller and less closely crowded fragments, and here size differences between adjacent nuclei were seen.

Table III *Prospective Cytologic Diagnoses and Histologic Confirmations for Three Cases of Endocervical Dysplasia (Group II)*

Case no	Cytologic diagnosis	Histologic confirmation	Date of diagnosis
16 (FL)	Invasive adenoca	Endocervical dyplasia	2/77
18 (MC)	AIS + microadenoca	Endocervical dysplasia	7/77
21 (GR)	Endocervical dysplasia	Endocervical dysplasia	8/78

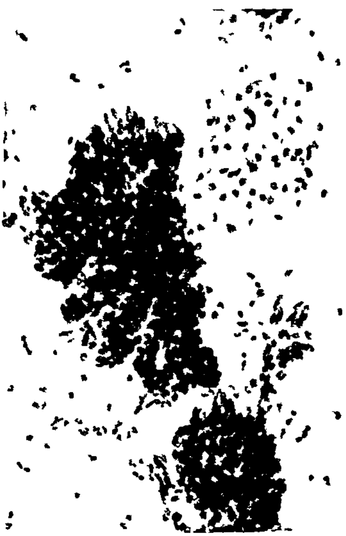


Figure 5
Case 21. Crowded sheets of cells from endocervical dysplasia (Papanicolaou-stained cervical scrape, × 180).

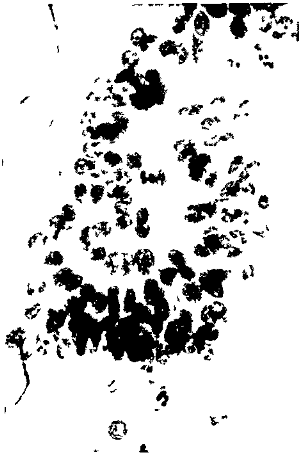


Figure 6
Case 21 Glandular fragment from endocervical dysplasia showing incipient pseudostratification (Papanicolaou-stained cervical scrape × 450)

Nuclear Shape The chromatinic membrane was smooth and evenly stained and the nucleus was round or oval in sheets viewed laterally

Chromatin Pattern The chromatin was fine and evenly granular Chromasia varied from mild to moderate hyperchromasia with mild hyperchromasia predominating

Nucleoli Small rounded nucleoli were commonly seen in the sheets and fragments of endocervical dysplasia

Endocervical Dysplasia in Inflammation An inflammatory reaction was evident in smears from the two cases in which endocervical dysplasia was diagnosed as either microinvasive or invasive adenocarcinoma In the case thought to show microinvasion (case 18) prominent smooth round macronucleoli were commonly present in cells that were otherwise similar in their cytologic presentation to those described above from noninflammatory smears Prominent smooth round macronucleoli were also present in the case in which invasive adenocarcinoma was expected (case 16) In addition the chromatin pattern here ranged from moderate to coarse granulation and there was a notable size variation between adjacent nuclei particularly in the less crowded sheets (Figure 7)

Group 3 Microinvasive Adenocarcinoma

Diagnostic Accuracy Microinvasion was accurately predicted in 4 of the 19 cases (Table IV) Invasive

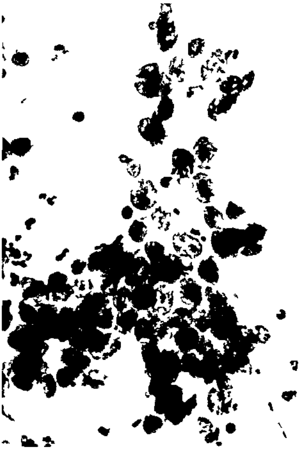


Figure 7
Case 16 Inflammatory changes in a less obviously glandular sheet of endocervical dysplasia Note prominent nucleoli coarse chromatin and nuclear size variation (Papanicolaou-stained cervical scrape × 450)

Table IV Prospective Cytologic Diagnoses and Histologic Confirmations for 19 Cases of Microinvasive or Early Invasive Adenocarcinoma (Group III)

Case no	Histologic confirmation	Date of diagnosis	False negative smears (year and grade)
Prospective diagnosis microinvasive adenocarcinoma			
23 (HW)	Microadenocarcinoma	5/75	1967 (endocervical dysplasia) 1970 '72 '73 '74 (AIS)
40 (TM)	Microadenocarcinoma	3/77	1969 (AIS) 1969 (AIS)
45 (JW)	Microadenocarcinoma	2/78	1970 '72 73 74 (AIS)
47 (SG)	Microadenocarcinoma	12/78	
Prospective diagnosis invasive adenocarcinoma			
43 (CK)	Microadenocarcinoma	12/77	
51 (AJ)	Microadenocarcinoma	5/78	
53 (SM)	Microadenocarcinoma	5/78	
Prospective diagnosis AIS			
22 (MN)	Microadenocarcinoma	6/74	
24 (VH)	Microadenocarcinoma + squamous atypia	7/75	1966 (AIS) 1972 73 (AIS)
30 (ED)	Microadenocarcinoma	10/75	1964 65 (AIS)
31 (MW)	Microadenocarcinoma	12/75	
50 (JW)	Microadenocarcinoma + squamous atypia	3/78	
Prospective diagnosis AIS + squamous atypia			
25 (GC)	Microadenocarcinoma + squamous atypia	10/74	
34 (LP)	Microadenocarcinoma + squamous atypia	5/76	
52 (DL)	Microadenocarcinoma + squamous atypia	4/76	
Prospective diagnosis squamous atypia only			
26 (BM)	Microadenocarcinoma + squamous atypia	5/73	
32 (JH)	Microadenocarcinoma + squamous atypia	1/76	
38 (SG)	Microadenocarcinoma + squamous atypia	1/77	
44 (VW)	Microadenocarcinoma + squamous atypia	1/78	

Squamous atypia also predicted

adenocarcinoma was expected in another three and five were undercalled as AIS In the remaining seven cases where squamous atypia was also predicted microinvasion was undercalled

Cytologic Observations In each of the four cases in which microinvasion was expected the smear background was noninflammatory, and nuclei were enlarged with prominent round oval or (in one case) irregular nucleoli The nuclear membrane was smoothly round or oval and the chromatin was moderate or coarsely granular Chromasia was variable and included a wide range from pale-staining nuclei with faint moderately uniform granulation to coarsely granular hyperchromatic nuclei Crowded and pseudostratified glandular sheets typical of the exfoliation pattern of AIS, were observed in two cases (23 and 45) and here the presence of prominent nucleoli provided the primary distinguishing feature of microinvasion In the other two cases (40 and 47) flat sheets with variable but often abundant amounts of cytoplasm separating adjacent nuclei were present in addition to sheets showing the structure of AIS (Figures 8 and 9) Nuclear atypicalities were most pronounced in these nonstratified sheets which also differed from those of a columnar morphology in regard to nuclear size There was a clearly discernible variation in the size of nuclei within these sheets as compared to the more uniform nuclear size of crowded and pseudostratified sheets

The three cases in which invasive adenocarcinoma was expected provided little opportunity for meaningful review Marked degenerative changes characterized the atypical material and nuclear details were only poorly preserved However on review of current smears of three of the four cases in which AIS rather than microinvasive adenocarcinoma was expected nonstratified squamoid sheets with prominent nucleoli and nuclei of various sizes with moderate or coarse chromatin granularity were seen These sheets were rare and nuclei were often faintly stained so the distinctive nuclear features were not immediately apparent Identification of this material in the company of AIS would require careful scrutiny

The seven cases in which microinvasive adenocar

Figure 8
Case 40 Destratified sheet of microinvasive adenocarcinoma material with nuclear criteria similar to those seen in inflammatory material from endocervical dysplasia (Papanicolaou-stained cervical scrape × 450)

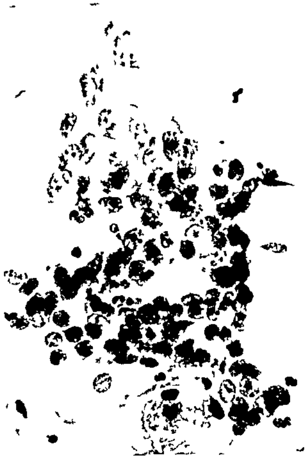


Figure 9
Case 47 Destratified sheet of microinvasive adenocarcinoma material Note the area at the top of the illustration showing abundant rather squamoid cytoplasm (Papanicolaou-stained cervical scrape × 450)

cinoma was undercalled in the presence of squamous atypia were also reviewed and in three of these cases material more probably representative of the squamoid glandular sheets seen in pure microinvasive adenocarcinoma rather than some form of squamous atypia was found This material would probably have been overlooked where AIS and squamous atypia were present concurrently

Group 4 Deeply Invasive Adenocarcinoma

Invasion was prospectively diagnosed in 6 of the 11 deeply invasive adenocarcinomas reported (Table V) The other five cases were undercalled as AIS The cytologic features of the accurately diagnosed cases are outlined below and an analysis of the underdiagnosed cases follows

Accurately Diagnosed Cases Well-differentiated sheets of columnar cells were *not* the salient cytologic features of the six cases where invasion was predicted even though four were reported histologically as well-differentiated adenocarcinoma one was moderately well differentiated and one showed adenosquamous differentiation

Abnormal cell groupings indicative of poorly differentiated adenocarcinoma only were seen in the smears of one case of well-differentiated adenocarcinoma (39) A small area of rather poorly differentiated adenocarcinoma was found in fact in the histologic material on review and cells from this area may have predominated in the smears although most of the lesion was well differentiated histologically In three other cases (33 29 and 36) small

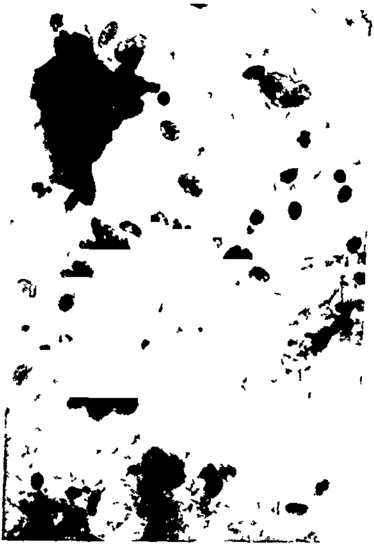


Figure 11
Case 35 Indeterminate group of cells from a deeply invasive adenocarcinoma Note the size relationship to adjacent intermediate cell nuclei and the irregular edge of the group where denuded nuclei project Crowding effects obscure further nuclear detail (Papanicolaou-stained cervical scrape × 450)

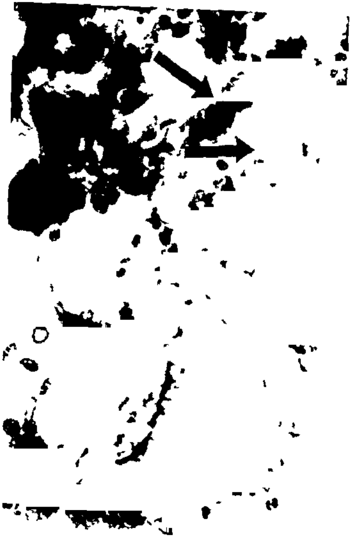


Figure 12
Case 35 A well-differentiated component of the same cervical smear from which Figure 11 was obtained (× 450) The closely crowded nuclei are equivalent in size to those shown in Figure 11 but show columnar structure at the sheet edges (arrows)

ly inflamed smears

Uniformity of nuclear size and shape within sheets was an important feature of AIS Contrary however to previous observations based upon a much smaller number of cases[4] nuclear enlargement was not a primary diagnostic feature Sheets of AIS comprising cells no larger than normal endocervical were a frequent and in one case predominant component of smears Similarly small-cell material also occurred in false negative smears and this could well have escaped notice whenever cell size was considered to be a more important parameter of AIS than was the exfoliative pattern

Pseudostratification and nuclear crowding was less well developed in noninflammatory smears showing endocervical dysplasia Size differences between adjacent nuclei were often seen particularly in sheets showing minimal pseudostratification but here nuclear chromatin was only mildly hyperchromatic and the lesion was accurately diagnosed Sheets of endocervical dysplasia however were very much more affected by inflammatory change than their AIS equivalents Macronucleoli were prominent and in one case coarse granulation of nuclear chromatin was also noted These nuclear changes were best seen in the less crowded sheets showing some evidence of nuclear pleomorphism and for this reason endocervical dysplasia in the presence of inflammation was overdiagnosed The differential diagnosis between endocervical dysplasia and more advanced cervical lesions is therefore of importance although apart from the reference of Koss[3] to endocervical dys karyosis we could find no evidence that this term has been applied in the literature relating to precancerous lesions of the cervix uteri The term *epithelial dysplasia* however has commonly been applied to abnormalities of neoplastic potential seen in columnar epithelial cells of the rectum[2,7,9] and Yardley[9] separated rectal biopsies described as mild moderate or marked epithelial dysplasia from *in situ* lesions The descriptions of epithelial dysplasia by these authors apply to columnar cells and include architectural abnormalities such as crowding and pseudostratification The usage of the term *epithelial dysplasia* therefore parallels at the cellular level that applied to endocervical dysplasia of the cervix uteri where however more specific terminology is required to exclude confusion with squamous dysplasia

Cases described histologically as either microinvasive or early invasive adenocarcinoma were reviewed collectively for this study as an entity separate from AIS Precise histologic definitions of microinvasive adenocarcinoma as distinct from invasive adenocarcinoma are however difficult to apply[1,4,6] A rather heterogeneous range of stages of early invasion was therefore represented with this grouping and any retrospective cytologic study must allow for variations in the extent to which microinvasive criteria are likely to be represented in smears Only four cases in fact were found to show clearly nuclear criteria similar to those described by Ng and Reagan[6] in relation to microinvasive squamous lesions Two of these cases were of immediate interest however in that the most pronounced nuclear abnormalities occurred in those sheets where pseudostratification was lost and a superficially squamoid appearance was seen Closely similar sheets were identified retrospectively in several undercalled cases of pure microinvasive adenocarcinoma and more important in cases that also showed squamous atypia Indeed microinvasive adenocarcinoma was undercalled in all of those cases where squamous atypia was also present These findings indicate the need for deliberate scrutiny of destratified or squamous sheets in the company of AIS Additionally nonstratified sheets with nuclear changes resembling those of microinvasive adenocarcinoma were seen in inflammatory smears of endocervical dysplasia The diagnostic problem arising here appears to parallel that described by Ng[5] in relation to the differentiation of dysplasia and microinvasive squamous lesions

Eleven deeply invasive adenocarcinomas were included in this study because in each of them an AIS element was clearly evident cytologically The majority of cases were well differentiated histologically and there apparently was a close relationship between invasive and *in situ* components Good supportive evidence of the relationship between AIS and invasive adenocarcinoma was represented by one rare example of poorly differentiated (adenosquamoid) adenocarcinoma A previous false negative smear showed material similar to that seen in the smears from two cases of AIS that were seen histologically as rather poorly differentiated variants

Diagnostic accuracy was quite low for deeply invasive cases and this may have been due to the fact that despite the general histologic predominance of very well differentiated material these cells very rarely if ever revealed meaningful nuclear criteria in the available cytologic smears Sheets of well-differentiated cells indistinguishable from AIS on the basis of their exfoliation pattern were common in some cases but nuclear criteria were either those of AIS or were obscured by crowding effect The obliteration of nuclear detail was most pronounced within crowded sheets of small cells and this material was apparently overlooked repeatedly in our laboratory particularly in the area of missed invasive cervical adenocarcinomas A larger series of cases would be required in order to evaluate further the significance of these sheets of small cells It is nevertheless clear from observations so far that at least from the standpoint of cytologic appearances increases in cell size are not a necessary concomitant of deep invasion More important sheets of small cells and indeterminate clusters of small nuclei should receive particularly close attention when seen in conjunction with exfoliative patterns otherwise indicative of AIS

Acknowledgments

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References

1 Burghardt E Early Histological Diagnosis of Cervical Cancer Philadelphia WB Saunders 1973 pp 350-362

2 Cook MG Goligher MS Carcinoma and epithelial dysplasia complicating ulcerative colitis Gastroenterology 68 1127 1136 1975

3 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Philadelphia JB Lippincott 1979 pp 398 399

4 Krumins I Young Q Pacey F Bousfield L Mulhearn L The cytologic diagnosis of adenocarcinoma *in situ* of the cervix uteri Acta Cytol 1 3-0-3 9 1977

5 Ng ABP Cytology of microinvasive cervical carcinoma Presented at the International Tutorial on Clinical Cytology Melbourne Australia 1973

6 Ng ABP Reagan JW Microinvasive carcinoma of the uterine cervix Am J Clin Pathol 52 511 529 1969

7 Nugent WF Haggitt RC Coleher H Kutteruf GC Malignant potential of chronic ulcerative colitis Gastroenterology 76 1 5 1979

8 Qizilbash AH *In-situ* and microinvasive adenocarcinoma of the uterine cervix Am J Clin Pathol 64 155-170 1975

9 Yardley JH Keren OF "Precancer" lesions in ulcerative colitis Cancer 34 385-844 1974

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The American Society of Cytology will hold its 28th Annual Scientific Meeting in Boston Massachusetts November 4-8 1980 *The program will include cytology* workshops roundtable luncheon discussions a diagnostic cytology seminar *panels and the presentation of selected scientific papers* Continuing medical education credits have been approved for Category I of the Physician s Recognition Award of the American Medical Association For further information write to Warren R Lang M D *Secretary Treasurer* Health Sciences Center 130 South 9th Street Philadelphia *Pennsylvania 19107*

The Cytology of the Pouch of Douglas in Polycystic Ovarian Disease

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Cul de sac aspiration was performed for cytologic sampling in 137 cases of polycystic ovaries treated by wedge resection Fifty patients undergoing abdominal tubal ligations also underwent aspiration of the pouch of Douglas as a control group

The cytodifferential count in polycystic ovarian disease showed 30% to 40% mesothelial cells 15% to 20% polymorphonuclear leukocytes 15% to 20% lymphocytes 10% to 15% squamous cells and 1% to 5% histiocytes The corresponding count in the control group showed 15% to 20% mesothelial cells 20% to 25% polymorphonuclear leukocytes 15% to 20% lymphocytes 10% to 15% squamous cells and 1% to 3% histiocytes Cells exfoliated from the fimbrial end of the tube were encountered in most smear

Abnormal cells were diagnosed in seven cases of polycystic ovarian disease due to a coexistent neoplasm i e two dermoid cysts a carcinoid tumor a hilus cell tumor a simple serous cyst a pseudomucinous cystadenoma and endometriosis of the ovary All tumors were histologically diagnosed in the resected wedges of the ovaries

In 1935 Stein and Leventhal first described the syndrome of bilaterally enlarged and cystic ovaries associated with menstrual irregularity anovulation infertility hirsutism and lack of mammary development[6] The three current theories regarding the etiologic factors are pituitary hypersecretion deficiency of luteinizing hormone and distortion of the ovarian spiral arterioles The association of the so-called Stein Leventhal syndrome with ovarian tumors is well known Novak and Woodruff[4] reported that 10% of polycystic ovaries have coexistent dermoid cysts The presence of a carcinoid tumor in a dermoid cyst however is a rare occurrence This is a report of a combined cytohistologic study of polycystic ovarian disease The cytologic findings in the peritoneal fluid obtained through aspiration of the pouch of Douglas were correlated with the pathologic examination of the removed wedges of the polycystic ovaries

Materials and Methods

The study was conducted on two groups of cases The primary group consisted of 137 cases of polycystic ovaries collected from the gynecology clinic at Ain Shams University *Hospital during* the years 1972 to 1976 The clinical description of these cases is presented in Table I

The diagnosis of polycystic ovarian disease was made by a modified technique of pelvic pneumography We used an undercouch tube instead of the usual overcouch tube so as to use far focus *film dis*tance and to put all procedures under visual control

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Table I *Clinical Description of 137 Cases of Polycystic Ovaries Diagnosed by Pelvic Pneumography*

	No of cases	Percentage
Virgins	7	5 1
Age in years	Mean 26 30±6 50	—
Amenorrhea	96	70 1
Menorrhagia	13	9 5
Infertility	130	94 9
Hirsutism	23	16 8
Obesity	64	46 7
Hypertension	12	8 8

We considered the ovary polycystic if its size was two or more times the normal one (Figure 1)

Cul-de-sac aspiration was performed for cytologic sampling of the pelvic cavity in every case diagnosed as polycystic ovaries The samples were collected without anesthesia with the patient in the lithotomy position and with a no 15 sterile spinal needle No complications with the aspirations were noted The available fluid volume varied from one drop to 7 ml The aspiration was considered successful if the smear contained mesothelial cells The aspirated fluid was fixed directly on the slide and stained with Papanicolaou stain

A preliminary endometrial curettage was also performed under general anesthesia for all cases before deciding on the wedge resection of the ovaries

Bilateral wedge resection of the polycystic ovaries was the line of treatment in all cases The removed wedges were examined pathologically, and the results were correlated with the cytology of peritoneal aspirate

As a control group 50 patients undergoing abdominal tubal ligation also underwent aspiration of the pouch of Douglas Small ovarian biopsies obtained during the laparotomy were also examined pathologically to exclude any abnormal changes

Figure 1
Pelvic pneumograph of polycystic ovaries

Table II *Endometrial Picture in 137 Cases of Polycystic Ovaries*

Type of endometrium	No of cases	Percentage
Proliferative	79	60 8
Hyperplasia	35	26 9
Secretory	19	14 6
Carcinoma	4	3 1

Results

Group with Polycystic Ovarian Disease (137 Cases)

The endometrial pathology is described in Table II It was proliferative in 79 cases hyperplastic in 35 and secretory in 19 We encountered four cases of endometrial carcinoma The histopathologic description was moderately differentiated adenocarcinoma in two cases and localized adenoacanthoma in the other two (Figure 2)

The pathology of the removed ovarian wedges showed the microscopic features listed in Table III In our diagnosis of polycystic ovarian disease we followed the pathologic findings reported for the removed wedges The clinical presentations, together with the radiologic findings were enough to indicate surgical intervention but the final diagnosis of the disease was based on the pathologic observations

The ovaries had a tough thickened and sclerotic capsule underneath which lay a zone of several tiny cysts Mature or degenerating corpora lutea usually absent were encountered in only five cases, four with secretory endometrium and one with localized endometrial adenoacanthoma amid a secretory endo

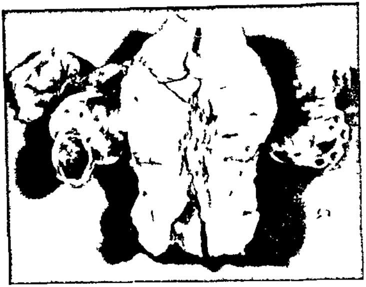

Figure 2
Localized endometrial adenoacanthoma with large polycystic ovaries

Table III *Microscopic Findings in 137 Cases of Polycystic Ovaries Treated by Bilateral Wedge Resection*

Description	Incidence (%)
Capsular thickening	
Marked	50
Slight	45
Absent	5
Cystic follicles	
Numerous	70
Few	25
Absent	5
Luteinization of cyst wall	
Present	60
Absent	40
Luteinization of stromal cells	
Present	30
Absent	70
Evidence of ovulation	
None	60
Old	40
Recent	0

metrium Secretory endometrium was reported in a total of 19 cases but we failed to encounter corpora lutea in the remaining 14 ovarian wedges

The prominent feature was the presence of many atretic follicles in various stages of maturation They were lined with thinned-out granulosa but had a remarkable hyperplasia of the theca cell layer (hyperthecosis) Luteinization of the theca cells was observed more often in cases of hirsutism Corpora fibrosa were present in most cases denoting involution and fibrosis of the atretic follicles Corpora fibrosa were absent in patients with endometrial carcinoma Few corpora albicantia denoting occasional previous ovulation were encountered in 19 patients including those with a history of previous pregnancy and those with secretory endometrium

In seven cases coexisting ovarian tumors were present They included two cases of dermoid cysts a carcinoid tumor a hilus cell tumor a simple serous cyst a pseudomucinous cystadenoma and endometriosis of the ovary (Figures 3 to 6) The aspirated fluid from the cul-de-sac was examined for its volume the different types of cells present and the cytodifferential count The available fluid volume varied from one drop to 7 ml but bore no relation to the phase of the menstrual cycle at the time of aspiration Mesothelial cells were present in all specimens (Figure 7) Other cell types encountered were polymorphonuclear leukocytes (PNL) lymphocytes erythrocytes histiocytes and squamous cells Cuboidal cells were seen in one case endometrial cells in another and a few clusters of mesothelial cells with nuclear enlargement and hyperchromatism in two cases Abundant squamous cells were observed in two cases and crystalloids in one case (Figures 8 and 9) Although endometrial cells may be encountered in the peritoneal aspirate especially during the first ten days of the menstrual cycle we did not come across endometrial cells except in the above reported case

5 Ng ABP Cytology of microinvasive cervical carcinoma Presented at the International Tutorial on Clinical Cytology Melbourne Australia 1973

6 Ng ABP Reagan JW Microinvasive carcinoma of the uterine cervix Am J Clin Pathol 52 511 529 1969

7 Nugent WF Haggitt RC Colcher H Kutteruf GC Malignant potential of chronic ulcerative colitis Gastroenterology 76 1 5 1979

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We examined the accuracy of pulmonary cytology in 224 consecutive patients being evaluated for lung cancer. The diagnostic yield of specimens obtained by various methods relating to flexible fiberoptic bronchoscopy (FFB) was compared.

Among 69 patients with lung cancer, a cytologic diagnosis was made in 87%, including 73% with peripheral tumors. Prebronchoscopy sputa were positive in 50%, bronchial washings in 63%, postbronchoscopy sputa in 72% and bronchial brushings in 50% of the patients. In only one patient was the bronchial brush specimen the only positive cytologic specimen.

Normal FFB and small cell undifferentiated cancer were found with increased frequency ($P < 0.05$) among the nine patients (13%) with false-negative cytology. Among 155 patients with nonmalignant cytology, 16 (10%) had false-positive specimens [illegible] lung disease. This finding was significantly related ($P < 0.05$) to necrotizing pneumonia in 13 of the 16 patients (81%).

The overall diagnostic accuracy of cytology showed 87% sensitivity and 90% specificity, and the predictive value of a positive specimen was 79%. In the absence of necrotizing pneumonia these values exceeded 95%.

The cytologic examination of pulmonary secretions has long been used as an adjunct to clinical and chest roentgenographic findings in the evaluation of patients suspected of having lung cancer. Despite the well-demonstrated diagnostic accuracy of expectorated sputum cytology, there remains considerable debate concerning whether the cytologic diagnosis of lung cancer should be confirmed by histologic [illegible] of biopsy material. This debate [illegible] of current inforrant initiation of drug therapy without surgical biopsy[1] Finally with the emphasis on the early detection of lung cancer the use of expectorated sputum cytology for screening purposes is under investigation and if cytology is positive vigorous diagnostic evaluation is frequently undertaken to detect *in situ* carcinoma even in the absence of clinical or chest roentgenographic abnormalities[13,33,34] Knowledge of the predictive value of positive sputum cytology would aid clinicians in establishing a reasonable plan of evaluation that would balance the diagnostic accuracy of these tests with the potential hazards of invasive procedures

The purpose of the present study was to determine prospectively the diagnostic sensitivity specificity and predictive value of pulmonary cytologic specimens obtained by various techniques in conjunction with FFB

Materials and Methods

A total of 224 consecutive patients who underwent diagnostic FFB at one institution during an 18 month period comprised the study population The primary indications for bronchoscopy were (1) persisting infiltrate seen on the chest roentgenogram 128 (57%) (2) hilar or parenchymal mass seen on chest roentgenogram 49 (22%) and (3) hemoptysis associated with a lung mass or infiltrate 47 (21%)

Bronchoscopic evaluation was performed by pulmonary fellows with the Olympus BF 5B flexible fiber optic bronchoscope (Olympus Corporation New Hyde Park New York) in a pulmonary procedures room where medications and equipment for resuscitation were available The bronchoscope was passed transnasally in all subjects

Multiple specimens for cytologic examination were collected using standardized methods processed and stained with Papanicolaou stain[38,48] Expectorated sputum was collected in a 50% solution of alcohol Sputa and bronchial washings were centrifuged and the sediment was used to prepare smears the remaining sediment was used for the preparation of a cell block Lesions visualized during bronchoscopy were brushed and direct smears were immediately immersed in 95% alcohol Fluoroscopic control for bronchial brushing was not available during the study period Transbronchial biopsies were not performed

Several cytotechnologists screened the cytologic specimens but one cytopathologist (A S) reviewed and interpreted all presumed abnormal specimens Specimens were classified as normal suspicious for malignancy or positive for malignancy using accepted criteria[25] Malignant cells were classified as epidermoid adenocarcinoma or undifferentiated In this report positive cytologic data refers only to specimens interpreted as consistent with malignancy the normal or suspicious specimens were combined and are referred to as negative cytologic data The cytologic interpretations were initially made without clinical information

The clinical records results of surgical biopsy procedures and autopsy data were reviewed A patient was considered to have had a false negative cytologic examination if all specimens submitted were negative for malignant cells and if cancer was confirmed by either biopsy or autopsy Patients were considered to have had false positive cytologic studies if one or more pulmonary cytologic specimens were interpreted as showing malignant cells but (1) repeat cytologic examinations at four month intervals were negative for malignant cells for a minimum of three years (2) initial chest roentgenographic findings suggesting cancer were resolved during follow up examinations or (3) extrapulmonary sources of malignant cells (esophagus and upper respiratory tract) were not present All false positive specimens were reviewed with available clinical information and were reclassified

Statistical comparisons were performed by chi square analysis with the Yates modification or Student t test for nonpaired data Probabilities equal to or less than 0 05 were considered significant The data were analyzed for specificity sensitivity and predictive value of a positive test using standard methods[32] These values were calculated using the initial and final cytologic interpretation

Results

The final diagnoses in the 224 patients examined were lung cancer 69 (31%) pulmonary bacterial or viral infection 76 (34%) pulmonary tuberculosis 40 (18%) chronic obstructive lung disease with atelectasis or nonspecific infiltrates 14 (6%) bronchiectasis 3 (1%) and other lung diseases 22 (10%) including sarcoidosis (6) fungal infection (5) pulmonary embolism (4) interstitial fibrosis (3) lung contusion (2) and bronchial adenoma (2)

The diagnosis of lung cancer was confirmed in 50 (72%) of 69 patients by histologic examination of tissue removed during the following procedures thoracotomy with lung biopsy or resection 20 (40%) bronchial biopsy through the rigid bronchoscope 16 (32%) cervical mediastinal exploration and lymph

Diagnostic Sensitivity and Specificity of Pulmonary Cytology

Comparison of Techniques Used in Conjunction with Flexible Fiber Optic Bronchoscopy

Stephen J Jay, M D
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We examined the accuracy of pulmonary cytology in 224 consecutive patients being evaluated for lung cancer The diagnostic yield of specimens obtained by various methods including flexible fiber optic bronchoscopy (FFB) was compared

Among 69 patients with lung cancer a cytologic diagnosis was made in 87% including 73% with peripheral tumors Prebronchoscopy sputa were positive in 50% bronchial washings in 63% postbronchoscopy sputa in 72% and bronchial brushings in 59% of the patients In only one patient was the bronchial brush specimen the only positive cytologic specimen

Normal FFB and small cell undifferentiated cancer were found with increased frequency ($P < 0.05$) among the nine patients (13%) with false negative cytology Among 155 patients with nonmalignant lung disease 16 (10%) had false positive specimens this finding was significantly related ($P < 0.05$) to necrotizing pneumonia in 13 of the 16 patients (81%)

The overall diagnostic accuracy of cytology showed 87% sensitivity and 90% specificity and the predictive value of a positive specimen was 79% In the absence of necrotizing pneumonia these values exceeded 95%

The cytologic examination of pulmonary secretions has long been used as an adjunct to clinical and chest roentgenographic findings in the evaluation of patients suspected of having lung cancer Despite the well-demonstrated diagnostic accuracy of expectorated sputum cytology [8-26] there remains considerable debate concerning whether the cytologic diagnosis of lung cancer should be confirmed by histologic examination of biopsy material [15-49] This debate stems largely from the relative lack of current information regarding the sensitivity and specificity of pulmonary cytologic data obtained by conventional methods in conjunction with flexible fiber optic bronchoscopy (FFB)

Several recent changes in the diagnostic evaluation and management of patients with lung cancer indicate an urgent need for this information First the development and widespread use of FFB often in lieu of expectorated sputum cytologic examination has not been accompanied by studies which compare the diagnostic yield of specimens obtained by various techniques [29-44] Second the recent development of chemotherapy protocols for the treatment of lung cancer has stimulated concern as to whether a positive sputum cytology is sufficient evidence to war

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rant initiation of drug therapy without surgical biopsy [1] Finally with the emphasis on the early detection of lung cancer the use of expectorated sputum cytology for screening purposes is under investigation and if cytology is positive vigorous diagnostic evaluation is frequently undertaken to detect *in situ* carcinoma even in the absence of clinical or chest roentgenographic abnormalities [13 33 34] Knowledge of the predictive value of positive sputum cytology would aid clinicians in establishing a reasonable plan of evaluation that would balance the diagnostic accuracy of these tests with the potential hazards of invasive procedures

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The diagnosis of lung cancer was confirmed in 50 (72%) of 69 patients by histologic examination of tissue removed during the following procedures thoracotomy with lung biopsy or resection 20 (40%) bronchial biopsy through the rigid bronchoscope 16 (32%) cervical mediastinal exploration and lymph node biopsy 7 (14%) extrapulmonary lymph node biopsy 5 (10%) and pleural biopsy 2 (4%) In 19 patients (28%) tissue was not obtained for histologic examination and the diagnosis of cancer was made on the basis of persistently positive sputum cytology in each patient and in addition by direct visualization of tumor through the flexible fiber optic bronchoscope in 12 subjects Fifteen of 19 patients died four months to three years following diagnosis and autopsies performed in eight revealed lung cancer Four patients were lost to follow up Four (6%) of 69 patients had primary extrapulmonary cancer metastatic to the lungs

A cytologic diagnosis of cancer was made in 60 (87%) of 69 patients In nine patients (13%) all specimens submitted for cytologic examination were negative for malignant cells (false negative) Twenty seven patients (39%) had one or more specimens that were interpreted as suspicious for malignancy Among patients with cancer 204 specimens (45%) were positive 42 (9%) were suspicious and 210 (46%) were negative for cancer Among 155 patients with nonmalignant lung disease 16 (10%) had one or more positive cytologic specimens (false positive) while the remaining 139 (90%) had negative cytologic evaluations Thirty four patients (22%) had suspicious cytology Among patients with nonmalignant lung disease 38 specimens (4%) were interpreted as positive 78 (8%) as suspicious and 833 (88%) as negative for cancer The cancer cell types determined during cytologic examination agreed with the cell type determined by histologic examination of tissue in 54 (94%) of 58 subjects A retrospective review with clinical correlation of false negative specimens revealed no change in the cytologic assessment Review of false positive specimens resulted in a change from the initial cytologic interpretation to suspicious in six cases and to negative in nine cases

A comparison of the diagnostic yield of various sources of cytologic material is shown in Table I During the initial phase of this study excess cytologic material was prepared as cell blocks Since the material from cell blocks did not significantly add to the cytologic diagnosis this procedure was discontinued Therefore the values in Table I represent the results of direct cytologic smears The mean number (and range) of cytologic specimens examined per patient was prebronchoscopy sputum (PB) 3 0 (range 0 to 9) bronchial washing (BW) 1 8 (range 0 to 6) and postbronchoscopy sputum (PBS) 3 7 (range 0 to 8) The diagnostic yield for PB was similar for central and peripheral tumors The distinction was based on whether lesions could have been seen during FFB with a rigid bronchoscope (central) or were located outside the visual range of the rigid bronchoscope

Table I *Bronchoscopic and Cytologic Findings in 69 Patients with Lung Cancer*

Histologic cell type and tumor location†	No of cases	%	Tumor visible through bronchoscope No of cases	%	Cytologic diagnosis Prebronchoscopy sputa (PB) No	%	Bronchial washing (BW) No	%	Bronchial brushing (BB) No	%	Postbronchoscopy sputa (PBS) No	%	All cytologic results negative No of cases	%
Epidermoid	42	61	30	71	23/34	68	27/42	64	22/30	73	30/38	79	3 42	7
Central	23	33	23	100	12/19	63	17/21	81	18/20	90	16/19	84	0	0
Peripheral	19	28	7	37	11/15	73	10/19	53	4/10	40	14/19	74	3/19	16
Undifferentiated‡	16	23	15	94	4/14	29	9/16	56	3/12	25	10 16	63	4/16	25
Central	8	11	8	100	2/8	25	4/8	50	1/5	20	4/8	50	3/8	38
Peripheral	8	12	7	88	2/6	33	5/8	63	2/7	29	6/8	75	1/8	13
Adenocarcinoma	11	16	6	55	2/10	20	6/11	55	4/7	57	7/11	64	2/11	18
Central	5	7	5	100	1/4	25	4/5	80	3/5	60	3/5	60	1/5	20
Peripheral	6	9	1	17	1/6	17	2/6	33	1/2	50	4/6	67	1/6	17
Total	*69*	*100*	*51*	74	29/58	50	42/67	63	29/49	59	47/65	72	9 69	13
Central	36	52	36	100	15/31	48	25/34	74	22/30	73	23/32	72	4 36	11
Peripheral	33	48	15	45	14/27	52	17/33	52	7/19	37	24/33	73	5 33	15

The values listed represent the number of patients with one or more positive specimens divided by the number of patients from whom satisfactory specimens were obtained for cytologic examination See text for average of specimens examined per patient

†Central tumors were defined as those that could have been visualized by rigid bronchoscopy peripheral tumors were defined as those beyond the visual range of the rigid bronchoscope

‡The undifferentiated group included small cell undifferentiated cancer (14 patients) and large cell undifferentiated cancer (2 patients)

(peripheral) [20] The yield of positive specimens from BW and bronchial brushings (BB) was higher for central lesions However the yield from PBS was similar for central and peripheral tumors The proportion of subjects with negative cytologic results was not significantly different among those with central tumors (4 of 36 11%) of peripheral tumors (5 of 33 15%) All sources of cytologic specimens demonstrated a high diagnostic yield in patients with epidermoid cancer This contrasts with the relatively low yield in patients with undifferentiated cancer or adenocarcinoma Postbronchoscopy sputa revealed the highest yield of any source and the yield was similar for all cell types

Direct visualization of tumor was achieved in 51 (74%) of 69 patients including 15 (45%) of 33 patients with lesions beyond the visual range of the rigid bronchoscope In 18 (26%) of 69 patients the tumors were beyond the visual range of the flexible bronchoscope and the yield of positive cytologic specimens in these patients was PB 7 of 16 (44%) BW 8 of 18 (44%) PBS 12 of 18 (67%) and BB 3 of 10 (33%) Bronchial brushing was carried out by advancing the brush blindly into the segmental bronchus presumed to be involved with cancer

The method of diagnosing lung cancer is shown in Table II Cytologic examination of either expectorated sputa or bronchial washings was diagnostic of cancer in 86% of the patients In one patient multiple sputa specimens were negative and only the bronchial brushing specimen revealed malignant cells It was uncommon for only one of the four sources of cytologic specimens to contain malignant cells i e only one patient by PB only three by BW only four by PBS only and one by BB only

None of the 69 patients with lung cancer had coexisting pneumonia We previously described the infrequent association of lung cancer and bacterial pneumonia [22]

Table II Method of Diagnosing Lung Cancer in 69 Patients

Method	Number of patients	Cumulative percentage
Cytologic		
Sputum	9	42
Bronchial washing	18	68
Postbronchoscopy sputum	12	86
Bronchial brush	1	87
Histologic		
Biopsy	2	89
Surgical resection	5	94
Autopsy	2	100

In 155 patients serial sputum cytologic examinations chest roentgenograms and clinical courses were consistent with nonmalignant lung diseases In 16 patients (10%) one or more cytologic specimens were initially interpreted as consistent with malignancy (false positive) In seven of these subjects one specimen was positive out of an average of eight (range four to nine) cytologic specimens examined per patient In nine subjects more than one specimen was positive (mean 3 8 range two to five) The total number of specimens examined in this group (mean 9 5 range two to 13) was similar to that of the former group A total of 38 false positive specimens was reported from these 16 patients this represents 4% of the total 949 cytologic specimens examined in the 155 subjects without lung cancer The source of false positive results was atypical squamous metaplasia that resembled squamous carcinoma (14 patients) and columnar cell hyperplasia which suggested adenocarcinoma (two patients) The false positive specimens were found with similar frequency in PS (10%) BW (8%) and PBS (10%) and with slightly lower frequency in BB (3%)

The characteristics of patients with false negative and false positive cytology are shown in Table III A normal bronchoscopic examination or one showing only extrinsic compression of airways and the presence of small cell undifferentiated carcinoma was found with greater frequency (P < 0 05) in patients with false negative results Several parameters did not differ in the true positive and false negative groups including age sex tobacco-smoking history

Table III Characteristics of Patients with False Negative and False Positive Cytology

False Negative Results	False negative (n = 9)		True positive (n = 60)		
	No of cases	Percentage	No of cases	Percentage	
Normal bronchoscopy	6	67	10	17	p = < 0 05
Undifferentiated small cell carcinoma	6	67	12	20	p = < 0 05
Epidermoid cancer	3	33	39	65	p = < 0 05

False Positive Results	False positive (n = 16)		True negative (n = 139)		
	No of cases	Percentage	No of cases	Percentage	
Acute lung infection	14	88	6	45	p = < 0 05
Active tuberculosis	1	6	39	28	p = < 0 05
Lung abscess	1	6	5	18	p = < 0 05

presence or extent of chronic obstructive lung disease or the number of cytologic specimens examined per patient

Patients with false-positive cytology were characterized by the presence of acute or resolving lung infection Six patients had necrotizing pneumonia (gram negative pathogens three *Nocardia asteroides* one *Actinomyces israelii* one *Staphylococcus aureus* one) Two patients had severe obstructive lung disease permanent tracheostomy and co existing bacterial pneumonia Two patients had severe obstructive lung disease and acute *Streptococcus pneumoniae* bronchopneumonia One patient had active cavitary pulmonary tuberculosis The remaining three patients had *Streptococcus pneumoniae* pneumonia and one of these patients had coexisting plasma cell dyscrasia Active pulmonary tuberculosis and lung abscess were present less frequently (P < 0 05) in the false positive group Again age sex tobacco-smoking history presence or severity of chronic obstructive lung disease and the number of cytologic specimens examined per patient were similar in the two groups

In 15 of 16 patients with false positive specimens follow-up sputum cytology was negative and sputum cytologic examinations at four month intervals remained negative In one patient with severe obstructive lung disease and a permanent tracheostomy sputa remained positive for the duration of follow up (three years) but the patient had not developed clinical chest roentgenographic or bronchoscopic evidence of lung cancer as of this writing Bronchial biopsy on two separate occasions revealed severe squamous metaplasia This patient may have carcinoma *in situ*

Follow up of patients with false positive specimens (mean 4 2 years) has not revealed evidence of lung cancer Twelve patients are alive and well Four patients died two in respiratory failure associated wit severe obstructive lung disease one from a myocardial infarction and one from renal failure Autopsie on two patients did not reveal lung cancer

The initial cytologic data were analyzed for th predictive value and accuracy of cytologic diagnosis These results are shown in Table IV The fraction o patients with lung cancer detected by positive cytology (sensitivity) was 87% the fraction of patients without cancer who were correctly identified by negative cytology (specificity) was 90% The predictive value of a positive cytologic result was 79% These values were based on overall results of cytology in individual patients

There were no complications of FFB or BB in the 224 patients investigated

Table IV *Sensitivity Specificity and Predictive Value of Cytologic Results*

Cytologic results	Lung cancer present	No lung cancer
Cancer	60 True positive (TP)	16 False positive (FP)
No cancer	9 False negative (FN)	139 True negative (TN)
Totals	69	155

Sensitivity = 87%
Specificity = 90%
Predictive value = 79%
Sensitivity (probability that cancer will be detected by the cytologic test) = TP TP + FN × 100 = (60 60 + 9) × 100 = 87%
Specificity (probability that a patient without cancer will have a negative cytologic evaluation) = TN TN + FP × 100 = (139 139 + 16) × 100 = 90%
Predictive value (probability that a positive cytologic test will accurately predict the presence of lung cancer) = TP TP + FP × 100 = (60 60 + 16) × 100 = 79%

Discussion

The clinical features of our patients were similar to those described in previous reports on the use of FFB in the evaluation of patients with suspected lung cancer[29,47,50] *This report differs from previous studies in* that we have systematically examined and compared cytologic results of expectorated sputa both pre- and postbronchoscopy with bronchial washings and brushings obtained during FFB

Our overall cytologic yield of 87% is similar to those of previous reports where rigid bronchoscopy was employed[8,26-35] It is also similar to those of recent reports that have included only cytologic data *from bronchial brushings performed through the* flexible fiber optic bronchoscope[9,29, 0,51] The excellent correlation *between the tumor cell type in cyto*logic specimens with the histologic cell type (94%) in the present study is consistent with previous reports[8,15,26,31]

Our proportion of positive cytologic specimens was higher regardless of the method used to obtain the samples in patients with central epidermoid tu*mors*[15,26,40] However of interest is that postbronchoscopy sputa showed the highest diagnostic yield *(> 70%) regardless of tumor cell type and regardless* of whether the lesion was central or peripheral in location In addition bronchial washings were positive in 63% of patients with lung cancer These findings are in sharp contrast to recent reports that concluded that bronchial washings and postbronchoscopy sputa should no longer be routinely used in the assessment of patients suspected of having lung cancer[29,44,45] In our study the only positive cytologic specimens were found in bronchial washings (three patients) and postbronchoscopy sputa (four patients) while all other specimens were negative. This represents a small (12%) but important diagnostic yield from these sources.[42,44]

Though we obtained a positive brush specimen in 91% of directly visualized central tumors, our yield with brushing peripheral tumors (37%) was considerably less than those of previous reports (> 50%) where fluoroscopic guidance was used.[9,28] Among 33 patients with peripheral cancers in the present study (Table I), 21 (64%) had lesions less than 3 cm in diameter on the chest radiograph. Positive cytologies were obtained from these patients from the following sources: prebronchoscopy sputa, 44%; bronchial washings, 43%; and postbronchoscopy sputa, 70%. In only two patients with masses less than 3 cm in diameter were all cytologic specimens negative. This represents the first systematic evaluation of the yield of sputum cytology in conjunction with FFB in patients with peripheral lung cancer; our results indicate that a relatively high diagnostic yield of cancer cells may be achieved without bronchial brushing under fluoroscopic control and transbronchial biopsy. The diagnostic yield of FFB and bronchial brushing and/or transbronchial biopsy has been recently examined in peripheral lung cancer with a reported diagnostic accuracy of 50% to 80%. However, the results of prebronchoscopy and postbronchoscopy sputum examinations and bronchial washings were not systematically evaluated and reported.[5,9,17,28] Our observations agree with a summary of ten earlier reports of studies in which FFB was not used that describe a surprisingly high (25% to 79%) diagnostic yield of sputum cytology in peripheral lung cancer.[8] Further studies are needed to clarify which patients should have more invasive procedures, such as transbronchial biopsy or transthoracic thin walled needle aspiration.[7,19] While bronchial brushing added little to the overall diagnostic yield in our study, we think that in view of the low complication rate[6,50] and excellent yield in small peripheral pulmonary lesions, the procedure should be used when placement of the brush into peripheral lesions can be achieved with biplane fluoroscopic guidance.[50,5] Transbronchial biopsy of peripheral lung cancer may have a greater diagnostic yield than bronchial brushing;[11,50] differences are slight, however, and the procedures seem to complement each other.[1,50] The morbidity and mortality of transbronchial lung biopsy appear to be sufficiently low[6,12,3,4,50] to employ it in selected patients. This procedure may also be useful in obtaining carinal biopsies for the purpose of staging lung cancer and helping determine operability.[3]

We have examined the characteristics of patients with false negative and false positive cytology. In patients subsequently shown to have lung cancer, nine (13%) had negative cytologic examinations. Other investigators have found a lower[8,30] or higher[8,26,42] incidence of false negative results. In studies where FFB has been used, the overall false negative cytologic results have been 4% to 28%.[29,42,49,50] The use of fluoroscopically guided bronchial brushing and transbronchial biopsy has improved diagnostic accuracy in some studies to over 75%.[20,45,50] Unfortunately, detailed results of sputum cytology are not presented in these studies, and it is therefore impossible to know in what fraction of patients a cytologic diagnosis of cancer was established before transbronchial biopsy was performed. False negative cytology has been attributed to multiple factors, including improper specimen collection,[10,26] sampling error,[8,10,35] peripheral location of the tumor,[6,8,10,35,42,51] small tumor size,[6,8,51] misinterpretation of cytologic smears,[2,26,30] examination of insufficient numbers of specimens,[8,26,30] bronchial obstructive lung tumor[8,10] and the presence of undifferentiated or metastatic cancer.[2,16,24,35] Among various parameters examined we found that only a normal bronchoscopic examination or extrinsic bronchial compression and the presence of small cell undifferentiated cancer were significantly ($p < 0.05$) associated with false negative results (Table III). The association of false negative cytology in undifferentiated lung cancer has been previously noted[2] and may relate in part to predominant lymph node involvement with this tumor.

The problem of false positive cytologic results is well recognized although the true incidence is difficult to determine because of nonuniform reporting by investigators. In a review of 20 published reports, 76 (3%) of 2,539 patients had presumed false positive cytology.[8] Recent studies utilizing FFB and bronchial brushing have reported that from 0.3% to 5.0% of patients have false positive specimens.[14,16,29,50,51] The variability in the incidence of false positive cases among otherwise comparable studies may reflect the variable experience of the cytopathology laboratory personnel. In nine series each with fewer than 100 cases of lung cancer the incidence of false positive cases was 5.3%, while in four studies each with more than 250 cases the rate was 2.9%.[8] In cases where the initial impression of malignant changes is sustained even with a review of false positive cytologic speci mens over 60% of specimens are found in patients with acute or chronic inflammatory disease of the lungs[8] In the present study 14 of 16 patients (88%) with false positive cytology had acute lung infections (Table III) and the major source of misinterpretation was the presence of atypical squamous metaplasia[8,27] We think this relatively high incidence of false positive results was due in part to the fact that specimens were interpreted initially without clinical history A retrospective review of these slides after a review of the clinical histories resulted in reclassifying nine patients as having negative cytology and six patients as having suspicious cytology

Bronchial epithelial cell atypia and metaplasia in lung infection are nonspecific cytologic changes that have been noted following irradiation of the chest[10,27] exposure to air pollutants[37] including uranium dust[41] and in association with nonpulmonary neoplasms[4] and interstitial lung disease[23] The absence of prolonged follow up data in previous reports on patients suspected of having false positive cytology has made it difficult to eliminate the possibility of *in situ* lung cancer[16,33,34] The prolonged period of follow up in our patients (mean 4 2 years) makes this possibility unlikely

Among patients with false positive cytology the majority had a low false positive to true negative ratio of about one to six In two patients false-positive specimens contained more than 50% of the specimens examined Both patients had permanent tracheostomy which has been associated with severe atypia and metaplasia of respiratory epithelial cells[43] The low frequency of false positive specimens to total specimens examined in individual patients contrasted with the high proportion (approximately 50%) of cytologic specimens that were positive in patients with proven lung cancer These findings may be helpful in identifying patients with false positive cytology

An important question addressed by this study was whether the sensitivity and specificity of lung cytology obtained in conjunction with FFB would justify the use of a cytologic diagnosis of cancer without tissue confirmation as a basis for definitive treatment with either radiotherapy or chemotherapy The term *sensitivity* indicates the fraction of patients with lung cancer who will be detected by sputum cytologic examination The *specificity* of sputum cytology reflects the fraction of patients who will be correctly identified as not having lung cancer The calculation of these param[illegible]s requires knowledge of the frequency of false p[illegible]d false negative cytologic results[32] Under ideal circumstances sputum cytology would always detect the presence of lung cancer—i e have a sensitivity of 100%—and would never be positive in patients without lung cancer—i e have a specificity of 100% The sensitivity and specificity of cytologic results in this study were approximately 90% with a 79% predictive value for a positive result (Table IV) In view of the strong association between false positive cytology and necrotizing pneumonia we recalculated these parameters after eliminating patients with pneumonia The specificity and predictive value of cytologic results were then 98% and 95% respectively We agree with a recent suggestion[19] that pulmonary cytology is a definitive diagnostic test and need not be routinely confirmed with a tissue diagnosis

Prospective multicenter studies are needed to answer the questions of what diagnostic tests should be employed to provide an accurate diagnosis of cancer with minimum expense and risk to patients and what methods should be used in evaluating operability and resectability Our data suggest that a combination of exfoliative sputum cytology in conjunction with FFB will answer these questions accurately and safely in over 85% of patients with lung cancer The role of procedures such as transbronchial and carinal biopsy cervical mediastinal node biopsy thoracoscopy radionuclide lung scans and computerized axial tomography used individually or in conjunction with other methods for the evaluation of suspected lung cancer remains to be determined

References

1 Aisner J Satterfield JR Aisner SC Pyeatte JC Wiernik PH Inflammatory changes related to active tuberculosis Confusion with oat cell carcinoma of the lung on cytology specimens Chest 73 670-671 1978

2 Bibbo M Fennessy JJ Lu C Straus FH Vanakogis D Wied GL Bronchial brushing technique for the cytologic diagnosis of peripheral lung lesions Acta Cytol 17 245 251 1973

3 Byrd RB Carr DT Miller WE Radiographic abnormalities in carcinoma of the lung as related to histological cell type Thorax 24 573 575 1969

4 Chalon J Katz JS Ramanathan S Ambiavagar M Orkin LR Tracheobronchial epithelial multinucleation in malignant disease Science 183 525 526 1974

5 Cortese DA McDougall JC Biopsy and brushing of peripheral lung cancer with fluoroscopic guidance Chest 75 141 145 1979

6 Credle WF Smiddy JF Elliott RC Complications of fiberoptic bronchoscopy Am Rev Respir Dis 109 67 72 1974 ✓

7 Dahlgren SE Lind B Comparison between diagnostic results obtained by transthoracic needle biopsy and by sputum cytology Acta Cytol 16 53 58 1972

8 Davies DF A review of detection methods for the early diagnosis of lung cancer J Chron Dis 19 819 845 1966

9 Dreisin RB Albert RK Talley PA Kryger MH Scoggin CH Zwillich CW Flexible fiberoptic bronchoscopy in the teaching hospital Chest 74 144 149 1978

10 Dulfano MJ Sputum Fundamentals and Clinical Pathology Springfield Illinois Charles C Thomas 1973 pp 397-400

11 Ellis JH Transbronchial lung biopsy via the fiberoptic bronchoscope Experience with 107 consecutive cases and comparison with bronchial brushing Chest 68 524 532 1975

12 Flick MR Wasson K Dunn L Block AJ Fatal pulmonary hemorrhage after transbronchial lung biopsy through the fiberoptic bronchoscope Am Rev Respir Dis 111 853 856 1975

13 Fontana RS Sanderson DR Woolner LB Miller WE Bernatz PE Payne WS Taylor WF The Mayo lung project for early detection and localization of bronchogenic carcinoma A status report Chest 67 511 522 1975

14 Funkhouser JW Meininger OE Cytologic aspects of bronchial brushing in a community hospital Acta Cytol 16 51 52 1972

15 Gagneten CB Geller CE Saenz MC Diagnosis of bronchogenic carcinoma through the cytologic examination of sputum with special reference to tumor typing Acta Cytol 20 530 536 1976

16 Grunze H Cytologic diagnosis of tumors of the chest Acta Cytol 17 148 159 1973

17 Hanson RR Zavala DC Rhodes ML Keim LW Transbronchial biopsy via flexible fiberoptic bronchoscope Results in 164 patients Am Rev Resp Dis 114 67 72 1976

18 Hattori S Matsuda M TV brushing method for early diagnosis of small peripheral lung cancer *In* Carcinoma of the Bronchus Edited by TJ Deeley New York Appleton Century Crofts 1971 pp 53-6

19 Hayata Y Ke k chi O Ichiba M Goya Y Hayashi T Percutaneous pulmonary puncture for cytologic diagnosis Its diagnostic value to small peripheral pulmonary carcinoma Acta Cytol 17 469-475 1973

20 Ikeda S Atlas of Flexible Bronchofiberscopy Baltimore University Park Press 1974 p 83

21 Ikeda S Flexible Bronchofiberscope Ann Otol Rhinol Laryngol 9 916 9 6 1970

2 Jay SJ Johanson WG Pierce AK The radiographic resolution of *Streptococcus pneumoniae* pneumonia N Engl J Med 293 798 801 1975

23 Kern WH Cytology of hyperplastic and neoplastic lesions of terminal bronchioles and alveoli Acta Cytol 9 372 379 1965

24 Kern WH Schweizer CW Sputum cytology of metastatic carcinoma of the lung Acta Cytol 0 514 5 0 1976

25 Koss LG Diagnostic Cytology and Its Histopathologic Bases Second edition Philadelphia JB Lippincott Co 1968 pp 3 5 379

26 Koss LG Melamed MR Goodner JT Pulmonary cytology A brief survey of diagnostic results from July 1st 195 until December 31st 1960 Acta Cytol 8 104 113 1964

7 Koss LG Richardson HL Some pitfalls of cytological diagnosis of lung cancer Cancer 8 937 947 1955

28 Kovnat DM Rath GS Anderson WM Siber F Snider GL Bronchial brushing through the flexible fiberoptic bronchoscope in the diagnosis of peripheral pulmonary lesions Chest 67 179 184 1975

29 Kvale PA Bode FR Kini S Diagnostic accuracy in lung cancer Comparison of techniques used in association with flexible fiberoptic bronchoscopy Chest 69 752 757 1976

30 Lopes Cardozo P DeGraaf S DeBoer MJ Doesburg N Kapsenberg PD The results of cytology in 1000 patients with pulmonary malignancy Acta Cytol 11 120 130 1967

31 Lukeman JM Reliability of cytologic diagnosis in cancer of the lung Cancer Chemother Rep 4 79 93 1973

32 McNeil BJ Keeler E Adelstein SJ Primer on certain elements of medical decision making N Engl J Med 293 211 215 1975

33 Melamed MR Zaman MB Flehinger BJ Martini N Radiologically occult in situ and incipient invasive epidermoid lung cancer Detection by sputum cytology in a survey of asymptomatic cigarette smokers Am J Surg Pathol 1 5 16 1977

34 Nasiell M Sinner W Tornvall G Roger V Vogel B Enstad I Clinically occult lung cancer with positive sputum cytology and primarily negative radiological findings Scand J Respir Dis 58 134 144 1977

35 Oswald NC Hinson KFW Canti G Miller AB The diagnosis of primary lung cancer with special reference to sputum cytology Thorax 26 623 631 1971

36 Pereira W Kovnat DM Snider GL A prospective cooperative study of complications following flexible fiberoptic bronchoscopy Chest 73 813 816 1978

37 Plamenac P Nikulin A Pikula B Cytologic changes of the respiratory tract in young adults as a consequence of high levels of air pollution exposure Acta Cytol 17 241 244 1973

38 Preece A Manual For Histologic Technicians Third edition Boston Little Brown & Co 1972 pp 172 179

39 Robbins HM Morrison DA Sweet ME Solomon DA Goldman AL Biopsy of the main carina Staging lung cancer with the fiberoptic bronchoscope Chest 75 484 486 1979

40 Rosa UW Prolla JC Gastal ES Cytology in diagnosis of cancer affecting the lung Results in 1000 consecutive cases Chest 63 203 207 1973

41 Saccomanno G Archer VE Auerbach O Saunders RP Brennan LM Development of carcinoma of the lung as reflected in exfoliated cells Cancer 33 256 269 1974

42 Saltzstein SL Harrell JH Cameron T Brushings washings or biopsy? Obtaining maximum value from flexible fiberoptic bronchoscopy in the diagnosis of cancer Chest 71 630-632 1977

43 Sara C Histological changes in the trachea and bronchi with tracheostomy Med J Austr 1 1174 1177 1967

44 Simmons DH Chopra S Fiberoptic bronchoscopy in bronchogenic carcinoma Chest 70 694-695 1976

45 Solomon DA Solliday NH Gracey DR Cytology in fiberoptic bronchoscopy Comparison of bronchial brushing washing and postbronchoscopy sputum Chest 65 616-619 1974

46 Suratt PM Smiddy JF Gruber B Deaths and complications associated with fiberoptic bronchoscopy Chest 69 747 751 19 6

47 Wanner A Amikam B Sackner MA A technique for bedside bronchofiberscopy Chest 61 287 288 1972

48 Woolner LB Cytologic preparation *In* Compendium on Diagnostic Cytology Second edition Edited by GL Wied LG Koss JW Reagan Chicago Tutorials of Cytology 1972 p 98

49 Yoneyama T Canlas MS From exfoliative" to diagnostic cytology A statistical evaluation of pulmonary cytology Acta Cytol 22 158 161 1978

50 Zavala DC Diagnostic fiberoptic bronchoscopy Techniques and results of biopsy in 600 patients Chest 68 12 19 1975

51 Zavala DC Richardson *RH Mukerjee PK Rossi NP Bedell* GN Use of the bronchofiberscope for bronchial brush biopsy Chest 63 889-892 1973

Indian Academy of Cytologists

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The highlight of the conference was Dr *Erica Wachtel*s guest *lecture Scope of Endocrine Cytology It stressed the limitations of evaluation of hormonal status by cytologic examination*

Dr K D Virkar s lecture Review of Vagino-cervical Cytology with Exogenous Estrogens and Progestin was the result of her painstaking work

The following were elected officers of the council for 1980

President	Dr D J Jussawala Bombay
Secretary	Dr Kamla Chandra Delhi
Treasurer	Dr P V Mehta Bombay
Members	Dr K D Virkar Bombay
	Dr J N Monga Gwalior
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Delegates to UICC (1979-82)	Dr Krishnamurthi Madras
	Dr *V Shanta Madras*

The next conference will be held in Hyderabad

Endoscopic Brush Cytology and Biopsy in the Diagnosis of Cancer of the Upper Gastrointestinal Tract

Ali H Qizilbash M D M I A C
Mario Castelli M D
Marie A Kowalski R T C T (IAC)
Anne Churly R T C T (ASCP)
C T (IAC)

Brush cytology and biopsy under direct vision was performed on 250 patients Of the 44 proven cases with cancers of the upper gastrointestinal tract brush cytology yielded positive results in 88 6% and multiple mucosal biopsies yielded positive results in 93 2% With the combined technique the results increased to 95 4% False negative results were due to large tumors with necrotic surfaces or to infiltrative tumors at the cardioesophageal junction There was one false positive with brush cytology and none with biopsy Four cases of early gastric cancer were diagnosed with the combined technique

The clinical application of gastrointestinal (GI) cytology in evaluating patients with symptoms of disease of the GI tract is well established Though cytology of the GI tract has been used over the years in the diagnosis of various benign conditions its primary role today still is in cancer detection[7] Over the past 15 years there has been remarkable progress in the various techniques used in the diagnosis and management of GI cancer The successful development of fiber optic endoscopy a technique whereby a lesion can be brushed and biopsied under direct vision has opened a new era in the evaluation diagnosis and management of lesions of the upper GI tract Not only are the newer techniques relatively simple to perform but results from the various centers attest to the high accuracy rate in the detection of cancer of the esophagus and stomach[3,4,6,8,12]

In a prospective study we assessed the value of endoscopic brush cytology and multiple biopsies in the diagnosis of cancer of the upper GI tract

Materials and Methods

The study began in the fall of 1975 with the opening of a new endoscopy unit at the Henderson General Hospital Hamilton Canada The first 250 cases in which endoscopic brush cytology and biopsy were performed form the basis of this report

Fiber optic forward and side viewing endoscopes were used in this study After the lesion was examined a small nylon brush was introduced via the endoscope and advanced to the lesion The suspicious area was brushed under direct vision The brushes were then smeared directly onto frosted slides and fixed immediately in 95% ethyl alcohol The smears were subsequently stained by the Papanicolaou technique Multiple biopsies were obtained at the same examination After examination of the lesion biopsy specimens were taken from the surface margins and surrounding mucosa of suspicious lesions They were fixed immediately in 10% buffered formalin Histologic sections were routinely stained with hematoxylin and eosin PAS/Alcian blue and reticulin stains

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Results

In 65 patients two brushings were employed one before and one after the biopsy The cytologic preparations were judged best before the biopsy in 15 cases and best after the biopsy in 30 cases Both brushings appeared essentially similar in 20 cases In the remainder of the patients only one brushing was performed

There were 44 cases of proven malignancy in this group of 250 patients Table I shows the type of cancers in this group of 44 patients Cytologic brush preparations were correctly reported as positive in 39 (88 6%) of the 44 proven cancers Negative results were obtained in four of the five cases of proven cancer of the stomach (Table II) In the fifth case spindle-shaped smooth muscle cells were present in the brush preparation but were interpreted as benign probably shed from a leiomyoma (Figure 1) This was the only case in the study where a false negative report was issued due to observer error

The only false positive diagnosis made in the study was on the cytologic smear from a patient with a pre pyloric ulcer The preparation contained very abnormal looking cells that were thought to represent adenocarcinoma cells (Figure 2) The cells were present in small groups and larger tissue fragments Many of the cells displayed variation in cell and nuclear size This variation was associated with chromatin clumping Single or multiple nucleoli were also present We were not confident of the diagnosis of carcinoma in this patient and requested a repeat examination in two weeks It was done and similar cells were not found on the repeat smear Gastric biopsies on both occasions did not reveal the presence of carcinoma The patient has been *followed for* over one year and the endoscopic and x ray findings have returned to normal

All patients underwent endoscopic biopsies Multiple specimens were usually taken the fragments were small varying between 1 and 2 mm in size and sometimes less Of the 44 cases with proven cancer the lesion was histologically interpreted as malignant in 41 (93 2%) *There were three false negatives* (Table II) Two of the three patients with negative biopsies had large ulcerated *tumors with necrotic surfaces* making tissue sampling difficult *In the third* recurrent adenocarcinoma at the gastroesophageal anastomosis had spread submucosally into the distal esophagus with stenosis *making adequate endoscopic* biopsies difficult to obtain There was no false positive diagnosis of *cancer on biopsy material in the* present study

If one includes the case of leiomyosarcoma with spindle-shaped tumor cells as positive then the combined brush biopsy results *increase* to 95 4% *in this* series of 44 cases of proven cancer of the upper GI tract

Discussion

Our experience with endoscopic brush cytology and biopsy reinforces the usefulness of the procedures in the diagnosis of cancer of the upper GI tract With the increasing use of endoscopy more and more cases of cancer are being diagnosed by this method which even in the elderly is safe relatively easy to perform and associated with little discomfort to the patient The results of our study and those of other investigators[4,6,8,1] confirm the high accuracy rate of *this technique in the detection of cancer of the upper* GI tract

The method of obtaining material under direct vision is superior to the *routine lavage cytology* Both brushing and biopsy can be done at the same

Table I Types of Cancer Upper GI Tract in 44 Patients with Proven Malignancies

	No of cases
Squamous cell carcinoma esophagus	3
Adenocarcinoma stomach	37
Leiomyosarcoma stomach	1
Lymphosarcoma stomach	1
Adenocarcinoma duodenum	2
Total	44

Table II *Malignancy of Upper GI Tract False Negative Cases*

Case no	Location and histologic type of lesion	Cytology	Biopsy
1	Recurrent adenocarcinoma gastroesophageal anastomosis	Negative	Negative
2	Infiltrative adenocarcinoma cardioesophageal junction	Negative	Positive
3	Linitis plastica carcinoma pylorus	Negative	Positive
4	Ulcerated adenocarcinoma fundus	Negative	Negative
5	Ulcerated leiomyosarcoma body	Negative†	Negative

Initial biopsy with fiber optic endoscope negative rebiopsy with rigid endoscope positive for carcinoma

†Smear contained malignant spindle-shaped cells *initially interpreted as* benign *error of interpretation* not of the technique

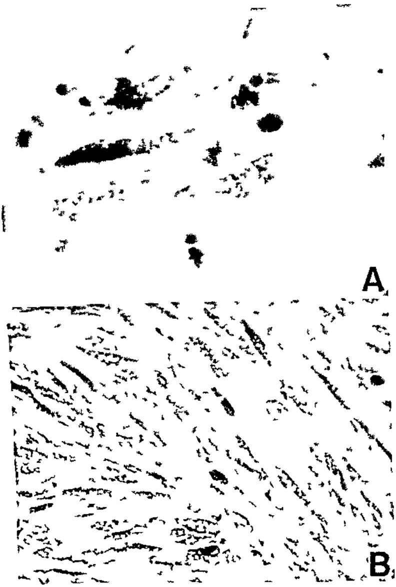

Figure 1
(A) Spindle-shaped smooth muscle cells from a case
leiomyosarcoma They were initially thought to repr
sent benign cells from a leiomyoma (Papanicolaou
stain oil × 1 250) (B) Resected stomach showing
spindle-shaped cells similar to the brush preparation
(hematoxylin and eosin × 500)

sitting With the brush technique the lesion can be visualized and vigorously scraped avoiding necrotic areas where possible The direct vision brushing technique has many advantages over the old ‘blind methods Cells can be obtained selectively from the suspicious areas under direct vision The smear can be prepared immediately withour prior centrifugation The smear is usually rich in cells and if the preparation is fixed immediately the preservation of cells is excellent In our experience these smears have a cleaner background and are far easier to screen than the blind lavage preparations

Multiple biopsy are usually obtained and in the case of ulcerated lesions biopsies should be taken from the floor of the ulcer as well as the inner edg and the margin [11] The number of biopsy specimen taken varies depending on the size and type of lesion but three to ten or more biopsies have bee recommended by various workers [1,2,4,10] Even in experienced hands only 40% to 50% of the biops fragments contain cancer [2,10] It is not unusual fo only one fragment to be malignant

In the present study the diagnostic accuracy c brush cytology was 88 6% and that of biopsy 93 2% With the combined methods the accuracy increased to 95 4% Our experience is similar to that c

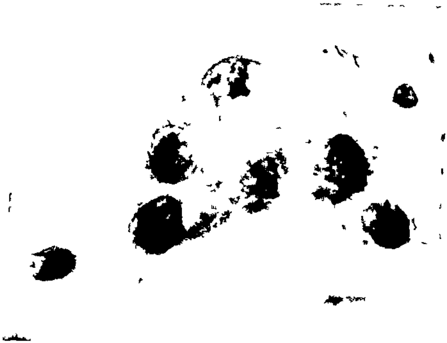

Figure 2
A group of cells from the edge of a benign ulcer showing marked atypical changes with prominent nucleoli They were wrongly interpreted as malignant (Papanicolaou stain oil × 1 250)

other workers [4 6 8 12 13] Biopsy yielded higher diagnostic accuracy than brush cytology Moreover there were no false positives on biopsy and even though the specimens obtained by this technique were small interpretation did not prove to be a diagnostic problem False negative results were due to infiltrative tumors or large ulcerated neoplasms that made tissue sampling difficult On the basis of our experience it appears that endoscopic biopsy should be the method of choice in the diagnosis of gastric cancer Brush cytology should be reserved for cases either where biopsy results are negative or a stricture prevents adequate tissue sampling Kasugai and Kobayashi[5 6] also recommend the use of direct vision gastric cytology when either the biopsy results are negative in the face of clinically suspicious lesions or a biopsy cannot be obtained

The differentiation of benign peptic ulcer from gas ric carcinoma on the basis of cytology poses no diffi ulty in the majority of cases However on occasion ven in experienced hands misinterpretation can oc ur This happened in one case in the present series Figure 2) It is not unusual and has been the experi nce of other authors Prolla et al[9] cite three such rrors in 524 cases of benign gastric ulcers studied ytologically Cells shed from gastric ulcers show a pectrum of changes from mild to severe atypia The ajority of atypias are of mild degree However oderate to severe changes do occur and they sually are the cause of errors The source of these ells is regenerating hyperplastic epithelium at the edge of the ulcer where enlarged cells with hyperchromatic nuclei and prominent nucleoli are present These cells usually shed in tissue fragments or large groups unlike the cancer cells which are present in smaller groups or clumps of individual cells Usually the cells associated with gastric ulcers have a fairly uniform size with little variation from one cell to the other The distinction however from well-differentiated adenocarcinoma may be difficult at times In contrast cells shed from a carcinoma show considerable pleomorphism and anisocytosis (Figure 3) Isolated glandular cells should be carefully examined for changes of malignancy because such single cells are nearly always found in association with sheets of tumor cells in such cases

The separation of metaplastic intestinal goblet cells from signet ring cancer cells may be difficult at times especially if occasional single cells are present Careful attention to the nuclear detail is important In cases of signet ring carcinoma the cells are more numerous presenting singly in small clumps or tissue fragments (Figure 4) Moreover variation in nuclear size and shape is seen even in the well-differentiated cancers The nuclei of metaplastic goblet cells on the other hand tend to be much more uniform

One case of leiomyosarcoma was present in this series *The brush preparation contained compact* groups of spindle to fusiform-shaped cells with delicate basophilic cytoplasm and cigar-shaped nuclei (Figure 1) Many bare nuclei were also noted The chromatin was finely granular and the nucleoli were

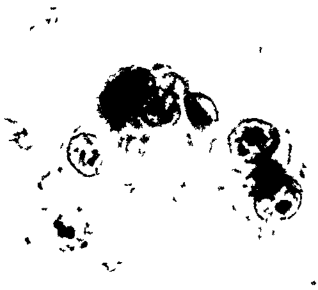

Figure 3
A group of cells from a case of well-differentiated adenocarcinoma There are parachromatin clearing large nucleoli and thickened nuclear membrane Compare with Figure 2 (Papanicolaou stain oil × 1 250)

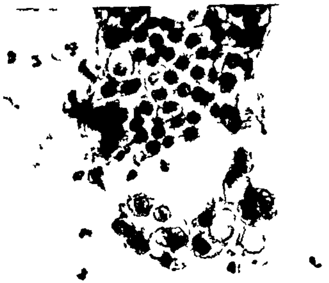

Figure 4
Signet ring adenocarcinoma cells contrasted with a she
of benign columnar cells (Papanicolaou stain × 500)

not very prominent Benign columnar cells and necrotic debris were also present They were recognized as of smooth muscle origin but the nuclear characteristics were not obviously malignant A cytologic diagnosis of leiomyoma was made The endoscopic biopsy consisted of necrotic debris and benign fragments of gastric mucosa The true nature of this lesion became obvious on examination of the resected stomach The histologic examination revealed a spindle-shaped tumor with numerous mitoses (Figure 1) The patient subsequently developed abdominal metastasis and died of the disease despit intensive chemotherapy The distinction between benign and malignant smooth muscle tumors is diff cult and even when multiple biopsies are studie definite evidence of malignancy may not be appa ent However it is important to recognize smoot muscle cells cytologically so that the lesion can b removed for thorough histologic examination

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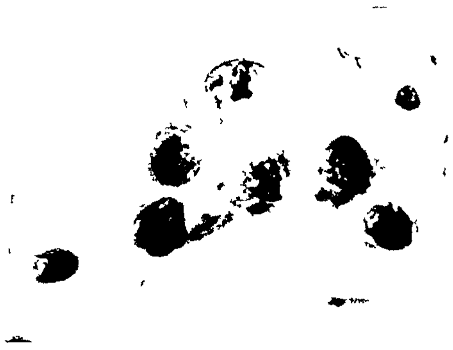

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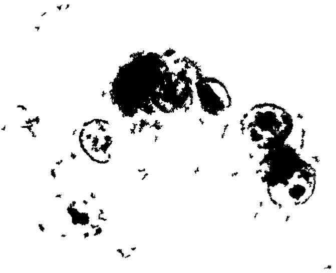

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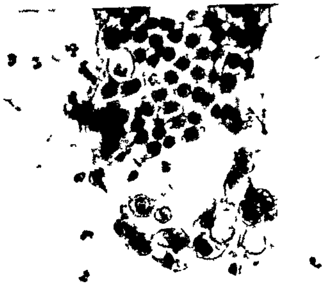
A group of cells from a case of well-differentiated adenocarcinoma. There are parachromatin clearing, large nucleoli and thickened nuclear membrane. Compare with Figure 2 (Papanicolaou stain, oil × 1 250)



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Of the 37 cases of carcinoma of the stomach, there were four examples of early gastric cancer. In these four patients both brushings and biopsies were per formed Malignant glandular cells were present in the brush preparations from three of the four patients the biopsies were positive in all four During the same period we saw three other cases of early gastric cancer in which endoscopic brushings were not per*formed Directed biopsies in all three* however revealed the presence of gastric cancer

References

1 Debray C Housset P Marche C le Parco CJ Verdier A Diagnosis of Stomach Cancer Today *In* Early Gastric Cancer Current *Status of Diagnosis Edited by E Grundmann H Grunze* S Witte Berlin Springer Verlag 1974 pp 120-126

2 Dekker W Tytgat GN Diagnostic accuracy of fiberendoscopy in the detection of upper intestinal malignancy A follow up analysis Gastroenterology 73 710 714 1977

3 Kameya S Nakamura S Mizutani K Hayakawa H Higashiyama S Kutsuna K Gastrofiberscope for biopsy Gastroenterol Endosc 6 36-40 1964 (in Japanese)

4 Kasugai T Kobayashi S Evaluation of biopsy and cytology in the diagnosis of gastric cancer Am J Gastroenterol 60 199 203 1974

5 Kasugai T Kobayashi S Kuno N Endoscopic cytology of the esophagus stomach and pancreas Acta Cytol 22 327 330 1978

6 Kobayashi S Kasugai T Brushing cytology for the diagnosis of gastric cancer involving the cardia or the lower esophagus Acta Cytol 22 155 157 1978

7 Prolla JC Kirsner JB Handbook and Atlas of Gastrointestinal Exfoliative Cytology Chicago University of Chicago Press 1972 pp 3-4

8 Prolla JC *Kelly KW Kirsner JB Cockerham L Direct vision* endoscopic cytology and biopsy in diagnosis of esophageal and gastric tumors Current experience Acta Cytol 21 399 402 1977

9 *Prolla JC Xavier RG Kirsner JB* Morphology of exfoliated cells in benign gastric ulcers Acta Cytol 15 178 182 1971

10 Qizilbash A Stevenson GW Early gastric cancer *In* Pathology Annual 1979 Part 1 Edited by SC Sommers PP Rosen New York Appleton-Century-Crofts 14 317 351 1979

11 Tamura K Masuzawa M Akiyama T Fukui O Touch smear cytology for endoscopic diagnosis of gastric carcinoma Am J Gastroenterol 67 463-467 1977

12 Winawer SJ Melamed MR Sherlock P Potential of endoscopy biopsy and cytology in the diagnosis and management of patients with cancer Clin Gastroenterol 5 575-595 1976

13 *Witte S Gastroenterological cytodiagnosis* Acta Hepatogastroenterol 22 137 139 1975

The Detection of Endometrial Tuberculosis in Cases of Infertility by Uterine Aspiration Cytology

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The cytology and histopathology of tuberculous endometritis are described in eight cases found among 200 women who presented with primary or secondary infertility Because tuberculous endometritis is considered a possible cause of such sterility the combination of endometrial aspiration and biopsy is recommended as an outpatient screening technique in such cases in areas where the rate of endometrial tuberculosis is high

Endometrial tuberculosis is not uncommon in India and may be a cause of secondary amenorrhea and infertility The lesion may be present even in the absence of any menstrual disorder or pelvic mass A simple outpatient procedure to detect endometrial tuberculosis is therefore necessary in all cases of infertility

There are many studies dealing with endometrial cytology in the detection of carcinoma of the body of the uterus but there are few studies relating to uterine cytology in the detection of endometrial tuberculosis[1,2,7,8] The present study was undertaken to determine the value of uterine cytology in the detection of tubercular endometritis in infertility cases Endometrial biopsy served as a control

Materials and Methods

Two hundred cases of infertility both primary and secondary were selected Routine investigations of infertility were performed on all couples Uterine aspiration and endometrial biopsy were done at an examination within one week of the next expected menses The aspiration was performed first followed by the biopsy The cannula used for aspiration was that devised by Cary Neither premedication nor anesthesia was necessary for cervical dilatation was not needed A drop of the aspirate was smeared on a slide and immediately fixed in 95% alcohol It was later stained with Papanicolaou stain The remainder of the aspirate was placed in a bottle containing 95% ethyl alcohol for preparation of the cell block The biopsy specimen was preserved in 10% formalin solution The cell block and biopsy specimen were stained with hematoxylin and eosin

Results

Of the 200 cases the aspiration as well as the biopsy material was inadequate for study in 10 cases Eight cases were considered suspicious for tubercular endometritis by uterine cytology and the diagnosis was confirmed histologically by either cell block or endometrial biopsy All eight cases had a raised erythrocyte sedimentation rate but none showed a lung lesion on X ray

Table I shows the menstrual pattern in the eight cases of tubercular endometritis One patient with normal cycles and complaining of abdominal pain was found to have bilateral tuboovarian masses and another with secondary amenorrhea had a small unilateral tuboovarian mass The remaining six appeared clinically normal

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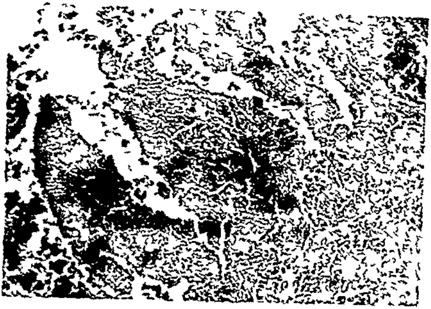

Figure 4
Eosinophilic granular material (hematoxylin and eosin × 450)

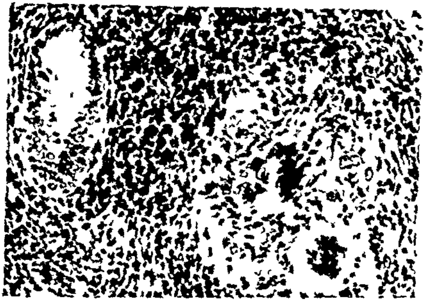

Figure 5
Langhans s giant cell granuloma in tubercular endometritis (hematoxylin and eosin × 450)

were diagnosed on the cell block alone the endometrial biopsy was inadequate in one and showed secretory endometrium in another with the smears of both showing only chronic inflammation One case was diagnosed by cell block and biopsy the smear showed chronic inflammation but no giant cells

Discussion

In the present study the incidence of tubercular endometritis was 4 2% This incidence is high as compared to that found in western countries but a high incidence of tubercular endometritis in India has been cited by other authors (Table IV)

The cytologic findings were essentially those of chronic inflammation and in one case eosinophilic material was seen Langhans s giant cells were seen in only four smears In the remaining three cases histology revealed the presence of giant cells The authors therefore conclude that chronic inflammation with epithelioid cells in the smear even in the absence of giant cells should raise the suspicion of tuberculosis in infertile women Similar observations have also been made by Coleman [2] Highman [4] Meisels and Fortin[5] and Misch et al [6]

Frost[3] occasionally found giant cells in postmeno-

Table III *Clinical, Cytologic and Histopathologic Picture of Endometrial Tuberculosis*

Case no	Age (years)	Type of infertility	Duration of infertility (years)	Complaints	Menstrual cycle	Pelvic findings	Aspiration cytology	Cell block	Endometrial biopsy
1	25	Primary	8	Infertility +	Amenorrhea (1 year)	Uterus retroverted, small size, fornices free	Chronic inflammation without giant cells	Noncaseating granuloma	Noncaseating granuloma
2	23	Primary	5	Infertility	Irregular, normal flow	Uterus anteverted, normal size, fornices free	Chronic inflammation with giant cells	Noncaseating granuloma	Noncaseating granuloma
3	28	Primary	12	Infertility	Amenorrhea (3 years)	Uterus retroverted, normal size, small tuboovarian mass in left fornix, right fornix free	Chronic inflammation with giant cells	Giant cells seen	Caseating tubercular endometritis
4	22	Secondary	5	Infertility	Oligoamenorrhea	Uterus anteverted, normal size, fornices free	Chronic inflammation with giant cells	Noncaseating granuloma	Noncaseating granuloma
5	17	Primary	2	Infertility +	Amenorrhea (10 months)	Uterus anteverted, normal size, fornices free	Eosinophilic granular caseous material only	Eosinophilic granular caseous material only	Caseating tubercular granuloma
6	35	Secondary	18	Infertility, pain in lower abdomen	Normal	Uterus anteverted, normal size, bilateral tuboovarian masses	Nonsecretory glands, chronic inflammation with giant cells	Noncaseating tubercular granuloma	Noncaseating tubercular granuloma
7	25	Secondary	5	Infertility	Normal	Uterus retroverted, normal size, fornices free	Secretory glands, chronic inflammation without giant cells	Noncaseating tubercular granuloma	Only secretory endometrium
8	29	Primary	8	Secondary amenorrhea	Amenorrhea (4 years)	Uterus anteverted, normal size, fornices free	Acute chronic inflammation without giant cells	Noncaseating tubercular granuloma	Inadequate

Table IV *Incidence of Tubercular Endometritis in India Cited in Previous Studies*

Author(s)	Incidence (%)
Ganguly (1972)	2.14
Panda and Dey (1972)	2.2
Saxena and Pathak (1972)	5.1

pausal smears, postradiation smears and *Trichomonas* infection. However, these giant cells differed from Langhans's giant cells of tuberculosis by their even distribution of nuclei and more definite cellular outline; at times they contained phagocytic debris. In addition, epithelioid cells in association with the giant cells are seen only in cases of tubercular endometritis.

Koss[7] and Terzano[11] reported tubercular smears that could have been mistaken for cancer. In advanced cases of tuberculosis, they found sheets of endometrial cells of uneven size with pronounced nuclear hyperchromasia suggestive of endometrial cancer. This was not observed in the present study.

Tubercular endometritis was missed by the biopsy in two cases: in one the tissue was inadequate, and the second had only secretory endometrium. The reason why tuberculosis should be picked up by aspira

Table I *Cases Without Initial Histologic Evidence of Hepatic Metastases*

Cytologic diagnosis	Histologic confirmation
1 Adenocarcinoma	Metastatic adenocarcinoma on repeat needle biopsy
2 Adenocarcinoma	Metastatic adenocarcinoma on *repeat needle* biopsy
3 Adenocarcinoma	Metastatic adenocarcinoma on repeat needle biopsy
4 Adenocarcinoma	Metastatic adenocarcinoma on open wedge biopsy tumor present in initial biopsy on reevaluation
5 Adenocarcinoma	Moderately well differentiated adenocarcinoma of the colon with hepatic metastases at autopsy three months later
6 *Large cell undifferentiated* carcinoma	*Poorly differentiated* adenocarcinoma with hepatic metastases at autopsy one year later
7 Undifferentiated malignant neoplasm	Poorly differentiated adenocarcinoma of the stomach with peritoneal metastases at time of surgery negative biopsy two months later autopsy not performed

cells were seen in the portal lymphatics which were not detected on initial evaluation Hepatic metastases were documented in two cases by autopsy One patient died three months after the liver biopsy Multiple nodules of moderately well differentiated adenocarcinoma of the colon were seen in the liver at autopsy Another patient had poorly differentiated adenocarcinoma of the prostate with hepatic metastases at autopsy Although this patient died one year after the liver biopsy was performed there was strong clinical evidence of metastatic carcinoma when the percutaneous biopsy was performed The patient had radiographic evidence of skeletal metastases an abnormal liver-spleen scan and elevated serum levels of alkaline phosphatase Despite the failure to demonstrate metastatic tumor in the biopsy a second attempt was considered unnecessary after the cytologic diagnosis supported the clinical impression Only one case was not confirmed by either autopsy or repeat biopsy This patient had poorly differentiated adenocarcinoma of the stomach with widespread peritoneal metastases at the time of surgery A liver biopsy was performed to attempt to demonstrate hepatic metastases two months later The patient died four months after the surgical resection and no autopsy was performed

In five of the nine cases in which neoplasms were detected by histologic sections only (Table II) well differentiated adenocarcinomas were present In another case infiltrates of chronic lymphocytic leukemia were recognized in the tissue sections Although lymphocytes were seen in the cytologic preparation they were well differentiated and a diagnosis of leukemia could not be established on this evidence alone One case of metastatic melanoma and one of small cell undifferentiated carcinoma were not detected by cytologic examination Review of these specimens demonstrated atypical cells but these single cells were too degenerate and infrequent to support an unequivocal diagnosis of malignancy on initial examination The cellular preservation was too poor for cytologic diagnosis in the only other case in which cancer was present in the biopsy but absent in the cytologic material

Discussion

Many methods have been advocated for the diagnosis of cancer in the liver These methods include clinical findings liver function tests liver scans and liver biopsy[3] Unlike the other methods which are largely inferential only biopsy can prove the presence of malignancy A single liver biopsy will successfully demonstrate cancer in 66% to 91% of patients subsequently proven to have hepatic metastases[2,5] The average of previously reported series is 71%[3] However Fenster and Klatskin have pointed out that the selection for biopsy of patients with overt clinical and laboratory evidence of hepatic metastases might explain the high degree of success in demonstrating tumor in the liver[4] Conn and Yesner attempted to

Table II *Cases Without Cytologic Evidence of Hepatic Metastases*

Histologic diagnosis	Cytologic diagnosis
1 Metastatic melanoma	No evidence of cancer
2 Chronic lymphocytic leukemia	Numerous mature lymphocytes
3 Small cell undifferentiated carcinoma	No evidence of cancer
4 Undifferentiated malignant adenocarcinoma	One atypical degenerate cell
5 Well-differentiated adenocarcinoma	No evidence of cancer
6 Well-differentiated adenocarcinoma	No evidence of cancer
7 Well-differentiated adenocarcinoma	No evidence of cancer
8 Well-differentiated adenocarcinoma	o evidence of cancer
9 Well-differentiated adenocarcinoma	No evidence of cancer

eliminate this observer bias and they were able to detect hepatic metastases in approximately half of their patients[3]

In previous similar series cytologic examination of fluid and touch preparations obtained at biopsy has proven to be a simple and effective method of increasing the sensitivity of the percutaneous liver biopsy in detecting hepatic metastases Sherlock et al[10] reported their experiences with 62 liver biopsies The 13 patients with carcinoma in histologic sections also had malignant cells in the smears Two patients with normal liver biopsies had malignant cells in cytologic specimen biopsies One of these patients had metastatic carcinoma at autopsy and the other had strong clinical and laboratory evidence of lymphomatous involvement of the liver Grossman et al[6] studied 67 patients with carcinoma 39 had hepatic metastases at autopsy when the biopsies were performed They found that a single biopsy detected 41% of cases two biopsies detected 54% and the addition of cytologic examination increased the yield to 69% The most recent experience has been reported by Carney[1] who examined 149 biopsies 27 of which contained histologic or cytologic evidence of cancer Eighteen cases had malignant cells in both the biopsy and smear In eight cases malignant cells were detected only in the smears Only a single case had cancer only in the histologic sections

In the present series 66 cases contained malignant neoplasms in either the biopsy or cytologic preparations In 50 of the cases malignant neoplasms were demonstrated by both methods Seven cases with normal liver biopsies had malignant cells in the cytologic preparation Nine cases with metastatic cancer in the biopsy were not detected by cytologic examination As in previous series cytology detected hepatic metastases which were not present in the biopsy Unlike the authors of those series we found a higher number of cases with cancer in the biopsy which was not present in the cytologic smears Previous reports have utilized cytologic smears prepared by rolling the tissue fragment across the glass slide Our specimens were prepared from the saline rinsings of the biopsy needle and therefore may have contained fewer cells Of the nine cases with malignant neoplasms only in the biopsy five were well-differentiated adenocarcinomas Perhaps the cellular exfoliation was lower in them than in less well differentiated neoplasms

Several explanations have been proposed for the occurrence of malignant cells in cytologic smears in the presence of negative biopsies Tumor cells may be present only on the surface of the tissue and remain within the needle when the tissue core is expelled[1] In addition the only viable tumor cells may be attached to the tissue fragment by a tenuous string of necrotic tumor which breaks off during the aspiration process[1] Grossman et al[6] emphasized that the biopsy needle passes through more tissue than is actually sampled Tumor cells may be detached into the bore of the needle as it passes through the lesion In four of their cases malignant cells were thought to be obtained from the pleural space lung or peritoneum In one of our cases the patient had widespread peritoneal metastases but no evidence of hepatic metastases It is conceivable that the malignant cells seen only in the cytologic material were from the peritoneum

From the current study we have concluded that the cytologic examination of liver biopsy specimens is a sensitive and extremely specific technique for detecting the presence of hepatic metastases When it is used in conjunction with routine histologic examination more diagnostic information can be obtained than by either method alone

References

1 Carney CN Clinical cytology of the liver Acta Cytol 19 244 250 1975

2 Conn HO Rational use of liver biopsy in the diagnosis of hepatic cancer Gastroenterology 62 142 146 1972

3 Conn HO Yesner R A re-evaluation of needle biopsy in the diagnosis of metastatic cancer of the liver Ann Intern Med 59 53-61 1963

4 Fenster LF Klatskin G Manifestations of the liver A study of eighty-one patients Am J Med 31 238 248 1961

5 Fisher CJ Faloon WW Comparison of initial clinical and histological diagnosis with a note on post-biopsy mortality in patients with metastatic neoplasm Am J Med 25 368 373 1958

6 Grossman E Goldstein MJ Koss LG Winawer SJ Sherlock P Cytological examination as an adjunct to liver biopsy in the diagnosis of hepatic metastases Gastroenterology 62 56-60 1972

7 Johansen S Myren J Fine-needle aspiration biopsy smears in the diagnosis of liver diseases Scand J Gastroenterol 6 583-588 1971

8 Lundquist A A fine needle aspiration biopsy for cytodiagnosis of malignant tumor in the liver Acta Med Scand 188 465-470 1970

9 Lundquist A Fine-needle biopsy of the liver Acta Med Scand [Suppl] 5 0 1 28 1971

10 Sherlock P Young YS Koss LG Cytologic diagnosis of cancer from aspirated material obtained at liver biopsy Am J Dig Dis 12 396-402 1967

Cytologic Findings in Postpartum and Postabortal Smears

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A study was undertaken to evaluate the cytologic features of pregnancy induced cell patterns (PIP) observed in postpartum and postabortal cervicovaginal smears A total of 3 000 PIP were evaluated and studied by an indirect immunofluorescent technique using human chorionic gonadotropin (HCG) Three characteristic types of PIP were demonstrated In type I PIP the cells had a sheetlike arrangement with a fine chromatin pattern and were thought to be of decidual origin Type II PIP were shed in clumps and had round nuclei cytoplasmic vacuoles and coarse chromatin They were thought to originate in the endometrium with the Arias Stella reaction Type III PIP were large and had large nuclei with marked atypism They were thought to be cytotrophoblasts Almost all of the type III PIP were positive with the anti HCG stain

It is concluded that this classification and analysis is significant and could reduce the number of false positive cytology reports on postpartum and postabortal smears

It is generally accepted that atypical endometrial cells may be obtained in postpartum and postabortal cervicovaginal smears However there are few definitive studies dealing with the features and behavior of these atypical cells The histologic characteristics of the endometrial changes are well established but there is only limited information regarding cytologic observations on the postpartum and postabortal states[13,24,25] The endometrial changes studied have included the Arias-Stella reaction in normal pregnancy[10,14,20] abortion[15,23] ectopic pregnancy[4,6,16,17,20] hydatidiform mole[20] and choriocarcinoma[4,9] The morphologic changes seen in the endometrium strongly resemble endometrial adenocarcinoma and various types of atypical cells were observed in postabortal cervicovaginal smears[9] Arias Stella reported that the histologic changes were induced by chorionic hormone and estrogen[3,5] however a meticulous study of the cytologic changes was not undertaken

The purpose of this study was to (1) describe the cytologic changes including the anti HCG staining of postpartum and postabortal cervicovaginal smears and (2) assess the morphologic alterations of the pregnancy induced cell pattern and determine their origin

Materials and Methods

Cervicovaginal smears from 528 postpartum or postabortal patients seen in the Department of Obstetrics and Gynecology of the Himeji Red Cross Hospital

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from January 1977 to August 1978 were examined for PIP A total of 47% of the postabortal patients and 21% of the postpartum ones showed PIP (Table I) Cytologic studies were carried out on the PIP positive cases Endometrial curettings were prepared in the usual manner for examination Cervicovaginal smears were stained with a modified Papanicolaou technique and the sections were stained with hematoxylin and eosin The cervicovaginal smears were obtained at least once a week until PIP disappeared

Immunofluorescence for Human Chorionic Gonadotropin (HCG)

For the demonstration of HCG cervicovaginal smears were stained with an indirect immunofluorescent technique [19] Tissue sections were prepared in the usual manner The sections were rinsed in xylene for ten minutes and in absolute ethanol for five minutes for deparaffinization

After formalin fixation the smears and sections were washed five to six times with phosphate buffered saline at a pH of 7 3 for ten minutes Rabbit anti HCG (Miles Yeda Ltd Israel) was placed on the specimens and they were incubated in a moist chamber at 37 C for 60 minutes The specimens were then washed sufficiently with phosphate buffered saline to remove the excess antiserum and were again incubated with fluorescein-conjugated goat antibody to rabbit immunoglobulin (Fujizoki Ltd Tokyo) at 37 C for 60 minutes Normal endometrium curettings from postpartum and postabortal women and normal rabbit serum were used as controls

Size Measurement and Configuration of PIP

The diameters of 3 950 cells and their nuclei were measured This included 3 000 PIP and 950 cells from endometrial adenocarcinoma The cells originating in the three types of PIP and endometrial adenocarcinoma cells were then evaluated for cell shape nuclear shape chromatin pattern and arrangements

Results

Classification of the PIP was made on the basis of the degree of morphologic alteration and three morphologic types were defined according to the following criteria Type I PIP were arranged in a sheetlike pattern with a polyhedral configuration and a fine nuclear chromatin They were thought to be of decidual origin (Figure 1) Their nuclear-cytoplasmic ratio was not increased Examination of an endometrial curetting specimen obtained one week after abortion disclosed decidual changes (Figure 2) These cells were similar in configuration to type I PIP seen in cervicovaginal smears (Figure 1)

Type II PIP were usually arranged in clusters and were isolated occasionally (Figure 3) Their nuclei were enlarged their chromatin was clumped and there was some variation in nuclear size Multiple small round nucleoli were often observed In the cytoplasm were many large vacuoles presumably related to mucus production in the tissue (Arias Stella phenomenon) These cells appeared histologically as the Arias Stella reaction seen in tissue sections (Figure 4)

Figure 1
Polyhedral exfoliated cells of type I PIP in a sheetlike arrangement There is granulated chromatin (Papanicolaou stain × 400)

Type III PIP had large nuclei with marked nuclear atypism These cells resembled trophoblastic cells seen in tissue sections (Figures 5 and 6) A clumping of these cells was observed frequently Almost all type III PIP were positive for immunofluorescent staining with anti HCG (Figure 7)

The incidence of PIP in the cervicovaginal smears of the 528 postpartum and postabortal patients is summarized in Table 1 Table I shows that 109 of 231 cases (47%) were positive for PIP smears and 122 (53%) were negative for PIP smears as classified on the basis of our criteria PIP were found in the smears of 61 of the 273 postpartum cases Table I also shows that PIP were more common in postabortal cases than in postpartum ones The time of detection of PIP is shown in Figure 8 In postabortal cases PIP

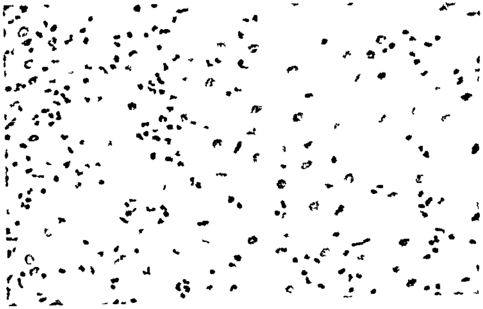

Figure 2
Histologic appearance of decidual reaction Note that stromal cells contain abundant cytoplasm with well-defined cell borders There is leukocytic infiltration (hematoxylin and eosin × 200)

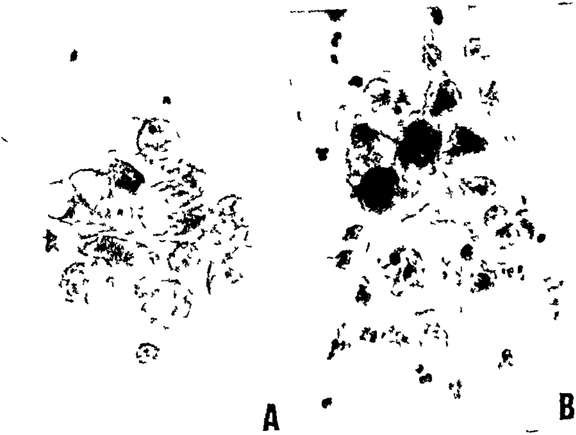

Figure 3
Exfoliated cells of type II PIP arranged in a cluster (A) There is nuclear enlargement chromatin clumping relatively large nuclei and large intracytoplasmic vacuoles (Papanicolaou stain × 1 000) (B) The nuclei are large and varied in size with irregular clumped chromatin (Papanicolaou stain × 1 000)

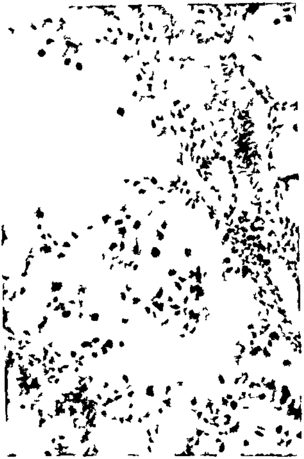

Figure 4
Tissue section of Arias Stella reaction in curettage specimen obtained six days after delivery. Note hypersecretory gland cells partly vacuolated with hyperchromatic nuclei (hematoxylin and eosin × 200)

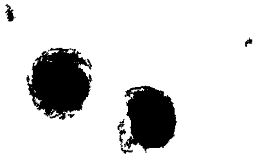

Figure 5
Exfoliated cells of type III PIP (cytotrophoblasts). The cells are round with well-defined cytoplasmic boundaries and have hyperchromatic irregular nuclei (Papanicolaou stain × 1 000)

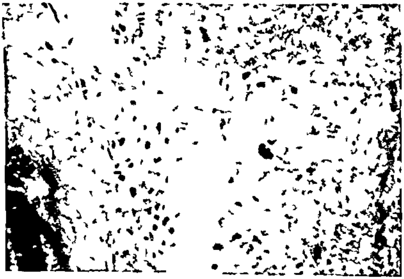

Figure 6
Tissue section of the curettage specimen obtained two weeks after abortion. Note hyperchromatic trophoblastic cells with enlarged nuclei in the decidua (hematoxylin and eosin × 100)

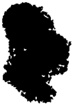

Figure 7
The type III PIP stain with anti HCG immunofluorescent technique (× 400)

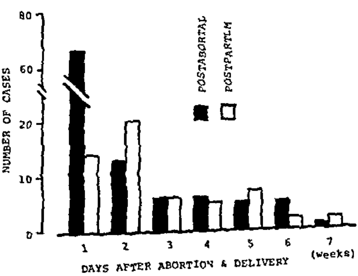

Figure 8
Time of detection of PIP in postpartum and postabortal cervicovaginal smears

Table I Incidence of Pregnancy Induced Cell Patterns (PIP) in Postpartum and Postabortal Cervicovaginal Smears

	Cases examined	PIP positive		PIP negative	
		No	%	No	%
Postpartum	297	61	21	236	79
Postabortal	231	109	47	122	53
Total	528	170	32	358	68

Table II Cell Configuration of Pregnancy Induced Cell Patterns (PIP) in Postpartum and Postabortal Cervicovaginal Smears

	Type I PIP (1 000 cells) (%)	Type II PIP (1 000 cells) (%)	Type III PIP (1 000 cells) (%)	Adenocarcinoma (9[illegible]0 cells) (%)
Cell shape				
Polyhedral	65	1	8	2
Spherical	30	88	13	84
Irregular	5	11	2	14
Nuclear shape				
Round	88	90	53	93
Ellipsoid	12	9	7	6
Irregular	0	1	40	1
Chromatin pattern				
Fine	65	5	2	64
Coarse	19	89	94	12
Opaque	16	6	4	24
Cell arrangement				
Single	10	5	60	21
Sheets	82	10	35	21
Cluster	8	85	5	58
Cell diameter (µm)	35 7±5 5	20 5±5 5	36 0±11 3	19 5±4 0
Nuclear diameter (µm)	12 5±6 5	17 5±4 3	12 3±13 2	13 5±4 6

occurred earlier than in postpartum cases Almost all the PIP appeared within one week in postabortal cases

The cell size and configuration of PIP from postpartum and postabortal women are summarized in Table II The cells of type I PIP have a round or ovoid nucleus with a fine chromatin network and a sheetlike arrangement These cells were only rarely weakly positive for anti HCG stain In type II PIP the nuclei had a coarsely granular chromatin and 85% occurred in clusters (Figure 3) The cells were negative for the anti HCG stain and were similar in configuration to the cells of adenocarcinoma Type III PIP were large and had large nuclei with coarsely granular chromatin A total of 60% of these cells were isolated and 35% were arranged in aggregates of three to five cells (Figure 5) Single round cells had well-defined cytoplasmic borders and a coarse chromatin pattern A total of 70% of the type III PIP stained positive for anti HCG (Figure 7) The relative frequencies of the three types of PIP in postpartum and postabortal cervicovaginal smears are summarized in Table III This comparison reveals that type I and type III PIP were common in both postpartum

Table III *Relative Frequencies of the Three Types of Pregnancy Induced Cell Patterns (PIP) in Postpartum and Postabortal Cervicovaginal Smears*

	Cases	Type I PIP		Type II PIP		Type III PIP	
	examined	No	%	No	%	No	%
Postpartum	61	25	41 0	6	9 8	30	49 2
Postabortal	109	35	3 1	17	15 6	57	52 3
Total	170	60	35 3	23	13 5	87	51 2

and postabortal cases Type II PIP occurred infrequently in both postpartum and postabortal cases totaling only 23 out of the 170 cases

Discussion

Since 1949 several reports have appeared on the cytologic features of postpartum and postabortal vaginal smears [8,9,24,26] Ferguson[9] stated that the glandular changes observed in the endometrium following abortion and their cytologic counterparts may result in false positive cytology Other well-established observations on atypical endometrial cells such as IUD related changes should be considered [11,12,21] The presence of atypical cells in postabortal and postpartum vaginal smears has been reported by several workers [7,9,13,24] *In summary most authors* agree that atypical cells may lead to errors in diagnosis on cervicovaginal smears

There still remains the problem of distinguishing the PIP from malignant tumor cells and other nonmalignant changes This study was done because there were no prospective studies dealing with the appearance of PIP in postpartum and postabortal cervicovaginal smears

Recently there has been a sharp rise in the frequency of endometrial neoplasia in the United States and in European countries *It is less apparent in Asia* Although a small number of endometrial adenocarcinomas were detected in cervical smears atypical cells resembling those in adenocarcinoma were common in postpartum and postabortal cervicovaginal smears Three morphologic variants of PIP were demonstrated and their tissue of origin is suggested These atypical cells included maternal and fetal elements corresponding to chorionic and decidual cells In type II PIP the nuclear and cytoplasmic features might be considered suspicious for or indicative of malignancy (Figure 3) Shrago[22] and Albukerk[1,2] showed that the cytologic changes of the Arias Stella reaction can be detected in cytosmears The type II PIP are similar to those reported by Shrago who also noted that many of the atypical endometrial cells were arranged in clusters of 5 to 20 cells The incidences of type II PIP are comparable in the postpartum and postabortal patient and the cells are almost always observed within two weeks following delivery or abortion (Figure 8)

In postpartum cases the cells exfoliate earlier than in postabortal cases when numerous trophoblastic cells remain in the endometrium (Figure 6) Type I and III PIP were found most often in postpartum and postabortal cases Syncytiotrophoblastic cells were easily recognized as type III PIP

There usually is little difficulty in distinguishing decidual cells from differentiated malignant tumor cells (so-called third cell type) [18] Isolated decidual *cells were sometimes difficult* to differentiate from single trophoblastic cells [7] but these were identified with the anti HCG stain Trophoblastic cells stained with anti HCG A few decidual cells were stained weakly with anti HCG No bizarre cell configurations were seen in decidual cells

These interrelationships of the endometrial regeneration to postpartum and *postabortal cytologic* findings may prove to have important practical applications

Acknowledgments

The authors are extremely grateful to James W Reagan M D F I A C for his helpful criticism and review of the manuscript *The authors would also* like to thank Isamu Sawaragi M D M I A C Yasuo Okada M D Hajime Okamoto M D Reginald K Anteson M D Francis X Makatia M T *M R S H* Toshiko Tsubakino M T and Hiroshi Kubo M T C T (IAC) for their outstanding contributions to this work

References

1 Albukerk J False positive cytology in the ectopic pregnancy N Engl J Med 291 1142 1974

2 Albukerk JN Gnecco CA *Atypical cytology in tubal pregnancy* J Reprod Med 19 273 276 1977

3 Arias Stella J Abnormal endometrial changes induced in the rat The effect of chorionic hormone and estrogen Arch Pathol 60 49 58 1955

4 Arias Stella J Atypical endometrial changes produced by chorionic tissue Hum Pathol 3 450-453 1972

5 Arias Stella J Endometrial changes in the rat The effect of estrogen when administered *after an interval following* castration Arch Pathol 60 59-6[illegible] 1955

6 Bermhardt RN Bruns PD Drose VE Atypical endometrium associated with ectopic pregnancy The Arias Stella phenomenon Obstet Gynecol 28 849-853 1966

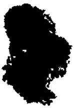

Figure 7
The type III PIP stain with anti HCG immunofluorescent technique (× 400)

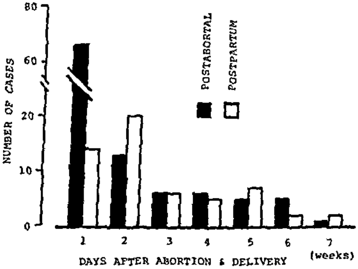

Figure 8
Time of detection of PIP in postpartum and postabortal cervicovaginal smears

Table I *Incidence of Pregnancy Induced Cell Patterns (PIP) in Postpartum and Postabortal Cervicovaginal Smears*

	Cases examined	PIP positive		PIP negative	
		No	%	No	%
Postpartum	297	61	21	236	79
Postabortal	231	109	47	122	53
Total	528	170	32	358	68

Table II *Cell Configuration of Pregnancy Induced Cell Patterns (PIP) in Postpartum and Postabortal Cervicovaginal Smears*

	Type I PIP (1 000 cells) (%)	Type II PIP (1 000 cells) (%)	Type III PIP (1 000 cells) (%)	Adenocarcinoma (950 cells) (%)
Cell shape				
Polyhedral	65	1	85	2
Spherical	30	88	13	84
Irregular	5	11	2	14
Nuclear shape				
Round	88	90	53	93
Ellipsoid	12	9	7	6
Irregular	0	1	40	1
Chromatin pattern				
Fine	65	5	2	64
Coarse	19	89	94	12
Opaque	16	6	4	24
Cell arrangement				
Single	10	5	60	21
Sheets	82	10	35	21
Cluster	8	85	5	58
Cell diameter (µm)	35 7±5 5	20 5±5 5	36 0±11 3	19 5±4 0
Nuclear diameter (µm)	12 5±6 5	17 5±4 3	1[illegible] 3±13 2	14 5±4 6

occurred earlier than in postpartum cases Almost all the PIP appeared within one week in postabortal cases

The cell size and configuration of PIP from post partum and postabortal women are summarized in Table II The cells of type I PIP have a round or ovoid nucleus with a fine chromatin network and a sheetlike arrangement *These cells were only rarely* weakly positive for anti *HCG stain In type II PIP the* nuclei *had a coarsely granular chromatin* and *85%* occurred in clusters (Figure 3) The cells were negative for the anti HCG stain and were similar in configuration to the cells of adenocarcinoma Type III PIP were large and had large nuclei with coarsely granular chromatin A total of 60% of these cells were isolated and 35% were arranged in aggregates of three to five cells (Figure 5) Single round cells had well-defined cytoplasmic borders and a coarse chromatin pattern *A total of 70% of the type III PIP* stained positive *for anti HCG (Figure 7)* The relative frequencies of the three types of *PIP in postpartum and postabortal cervicovaginal* smears are summarized in Table III This comparison reveals that type I *and type* III PIP were common in both postpartum

Table III *Relative Frequencies of the Three Types of Pregnancy Induced Cell Patterns (PIP) in Postpartum and Postabortal Cervicovaginal Smears*

	Cases examined	Type I PIP		Type II PIP		Type III PIP	
		No	%	No	%	No	%
Postpartum	61	25	41 0	6	9 8	30	49 2
Postabortal	109	35	32 1	17	15 6	57	52 3
Total	170	60	35 3	23	13 5	87	51 2

and postabortal cases Type II PIP occurred infrequently *in both postpartum and postabortal cases* totaling only 23 out of the 170 cases

Discussion

Since 1949 several reports have appeared on the cytologic features of postpartum and postabortal vaginal smears [8,9,24,2] Ferguson[9] stated that the glandular changes observed in the endometrium following abortion and their cytologic counterparts may result in false positive cytology Other well-established observations on atypical endometrial cells such as IUD related changes *should be considered* [1,12,21] The presence of atypical cells in postabortal and postpartum vaginal smears has been reported by several workers [7,9,13,24] In summary most authors agree *that atypical cells may lead* to errors in diagnosis on cervicovaginal smears

There still remains the problem of distinguishing the PIP from malignant tumor cells and other nonmalignant changes This study was done because there were no prospective studies dealing with the appearance of PIP in postpartum and postabortal cervicovaginal smears

Recently there has been a sharp rise in the frequency of endometrial neoplasia in the United States and in European countries It is less apparent in Asia Although a small number of *endometrial adenocarcinomas* were detected in cervical smears atypical cells resembling those in adenocarcinoma were common in postpartum and postabortal cervicovaginal *smears Three* morphologic variants of PIP were demonstrated and their tissue of origin is suggested These atypical cells included maternal and fetal elements corresponding to chorionic and decidual cells *In type II PIP* the nuclear and cytoplasmic features might be considered suspicious for or indicative of malignancy (Figure 3) Shrago[22] and Albukerk[1,2] showed that the cytologic changes of the Arias Stella reaction can be detected in cytosmears The type II PIP are similar to those reported by Shrago who also noted that many of the atypical endometrial cells *were arranged in clusters of 5 to 20 cells* The incidences of type II PIP are comparable in the postpartum and postabortal patient and the cells are almost always observed within two weeks following delivery or abortion (Figure 8)

In postpartum cases the cells exfoliate earlier than in postabortal cases when numerous trophoblastic cells remain in the endometrium (Figure 6) Type I and III PIP were found most often in postpartum and postabortal cases Syncytiotrophoblastic cells were easily recognized as type III PIP

There usually is little difficulty in distinguishing decidual cells from differentiated malignant tumor cells (so-called third cell type) [18] Isolated decidual cells were sometimes difficult to differentiate from single trophoblastic cells [7] but these were identified with the anti HCG stain Trophoblastic cells stained with anti HCG A few decidual cells were stained weakly with anti HCG No bizarre cell configurations were seen in decidual cells

These interrelationships of the endometrial regeneration to postpartum and postabortal cytologic *findings may prove* to have important practical applications

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5 Arias Stella J Endometrial changes in the rat The effect of estrogen when administered after an interval following castration Arch Pathol 60 59-62 1955

6 Bermhardt RN Bruns PD Drose VE Atypical endometrium associated with ectopic pregnancy The Arias Stella phenomenon Obstet Gynecol 28 849-853 1966

7 Danos M Holmquist ND Cytologic evaluation of decidual cells A report of two cases with false abnormal cytology Acta Cytol 11 325 330 1967

8 Fayad MM Youssef AF Vaginal cytology in clinically threatened abortion *J Obstet Gynaecol Br Commonw 70 39-45* 1963

9 Ferguson JH Some limitations of cytological diagnosis of malignant tumors Cancer 2 845-852 1949

10 *Fienberg R Lloyd HED The Arias Stella reaction in early normal pregnancy*—an involutional phenomenon The ovary placenta changeover as a possible cause Hum Pathol 5 183 190 1974

11 Fornari ML Cellular changes in the glandular epithelium of patients using IUCD A source of cytologic error Acta Cytol 18 341 343 1974

12 Gupta PK Burroughs F Luff RD Frost JK Erozan YS Epithelial atypias associated with intrauterine contraceptive devices *(IUDs) Acta Cytol 22 286 291 1978*

13 Hilrich NM Hipke MM Endometrial and cytologic atypism in the postabortal state Report of a case Obstet Gynecol 6 452-454 1955

14 *Holmes EJ Lyle WH Yuma AZ How early in pregnancy does* the Arias Stella reaction occur? Arch Pathol 95 302 303 1973

15 Holmquist ND Danos M The cytology of early abortion Acta Cytol 11 262 266 1967

16 Kreszury S Examination of curettage material for the diagnosis of ectopic pregnancy Acta Morphol Acad Sci Hung 24 359 367 1976

17 Lloyd HED Fienberg R The Arias Stella reaction A nonspecific involutional phenomenon in intra as well as extrauterine pregnancy Am J Clin Pathol 43 428-432 1965

18 Naib ZM Single trophoblastic cells as a source of error in the interpretation of routine vaginal smears Cancer 14 1183-1185 1961

19 Nairn RC Fluorescent Protein Tracing Fourth edition Edinburgh Churchill Livingstone 1976 pp 125-169

20 *Roach WR Guderian AM Brewer JI Endometrial gland cell* atypism in the presence of trophoblast Am J Obstet Gynecol 79 680-691 1960

21 Sagiroglu N Sagiroglu E The cytology of intrauterine contraceptive devices Acta Cytol 14 58-64 1970

22 Shrago SS The Arias Stella reaction A case report of a cytologic presentation *Acta Cytol 21 310-313 1977*

23 Silverberg SG Arias Stella phenomenon in spontaneous and therapeutic abortion Am J Obstet Gynecol 112 777 780 1972

24 Soloway HB Vaginal and cervical cytology of the early puerperium Acta Cytol 13 136-138 1969

25 Tanaka S Ikeda H Tsuchiya N Ueno T Tanaka N Sequential cytopathological study after D&C in pregnancy with special *reference to the atypical cells J Jpn Soc Clin Cytol 17 214* 219 1978 (in Japanese)

26 Youssef AF Fayad MM The post partum vaginal smear J Obstet Gynaecol Br Commonw 70 32 38 1963

Problems in Cervicovaginal Cytology

Fine Structure as an Aid to Diagnosis

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E Blanche Butler M D F I A C
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The ultrastructure of cytologically abnormal thick cell groups and epithelial fragments in cervical and vaginal scrape material was investigated and found to be useful in differentiating between carcinoma in situ *invasive nonkeratinizing squamous carcinoma and adenocarcinoma of the endocervix and also in confirming the presence of cytologically suspected vault deposits of recurrent endometrial adenocarcinoma*

It was demonstrated that although accurate evaluation of these thick groups in smear preparations is often not possible thick sections of similar plastic embedded material showed some features which enabled a distinction to be made between squamous and glandular lesions and that these differences were more pronounced at the ultrastructural level The squamous lesions were characterized by wide intercellular spaces with microvilli and tonofibrils within the cytoplasm while glandular lesions showed narrow intercellular spaces prominent Golgi zones and endoplasmic reticulum together with mucus droplets in some cells

In cervical smears fragments of normal epithelium (both squamous and columnar) and fragments showing mild to moderate dysplasia can be recognized because the nuclei are well separated and because it is possible to focus to different levels in the fragments In contrast when the constituent cells have a very high nuclear-cytoplasmic ratio the density of the group prevents an accurate evaluation of individual cells and this presents particular difficulty when there is no accompanying squamous cell dyskaryosis Even when the latter is present indicating a squamous lesion it may not be clear whether the lesion is carcinoma *in situ* or nonkeratinizing squamous carcinoma the possibility of a coexisting adenocarcinoma also has to be considered

This study was undertaken to evaluate the use of the electron microscope in identifying the types of cells which exfoliate in adherent groups and are present in cervical and vaginal scrape material

Materials and Methods

Many of the patients with abnormal cytology in St Mary s Hospital Manchester were examined colposcopically by the cytopathologists (E B B and C M S) and material could therefore be collected from those patients known to be exfoliating thick groups of abnormal cells as well as from those with more easily classified abnormalities

In this investigation cellular samples for electron microscopy were obtained from the cervices of ten patients for whom the cytologic diagnosis was in some doubt and from the vaginal vaults of three patients who had had hysterectomies for endometrial adenocarcinoma and whose smears suggested recurrent tumors in the absence of clinical abnormalities

Two samples were collected from each patient one with a wooden Ayre spatula from which a routine smear was first made and another with a plastic Armour spatula The shape of the Armour spatula is such that the central protuberance reaches well into

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the endocervical canal and so collects material from the lower endocervix It has been found that a more representative sample is obtained using the two spatulae *together than with either alone The two spatu*lae were placed together in a universal container of cold fixative any adherent material was removed by scraping one against the other

Fixatives used were either 2 5% glutaraldehyde in cacodylate buffer (pH 7 4) for a minimum of 90 minutes at 4 C or a mixture of 2% paraformaldehyde and 2% glutaraldehyde in cacodylate buffer for a minimum of four hours at 4 C After fixation the cells were centrifuged and the fixative replaced by cacodylate buffer The material was then held at 4 C and when convenient it was postfixed in osmium tetroxide dehydrated in an ascending series of alcohols and embedded in either Araldite or Taab resin Thick sections were stained with 1% toluidine blue for light microscopy and material containing groups of cells or fragments of epithelium was selected for electron microscopy Thin sections from these selected blocks were then prepared stained with uranyl acetate and lead citrate and examined in a Philips 301 electron *microscope (Philips Eindhoven Holland)*

The fine structure of similarly prepared biopsy material from four patients with carcinoma *in situ* was also examined in order to compare the morphology of cell and tissue samples

Results

The correlated colposcopic biopsy electron microscopic and histologic findings are shown in Table I

Normal Cells

In plastic-embedded sections (1μ) at light level fragments of normal superficial squamous epithelium groups of metaplastic cells and sheets or papillary fragments of endocervical epithelium were easily recognized Superficial squamous cells had small irregular nuclei and abundant cytoplasm often with

Table I Summary of Findings in 13 Patients Discussed in the Text

Case	Age	Colposcopy	Colposcopic biopsy	Fine structure	Histologic diagnosis
1 JK	26	Aceto white epithelium with mosaicism	*Mild dysplasia*	*CIS*	*CIN grades 2 & 3*
2 MW	42	*Atypical TZ with* punctation	None taken	CIS	CIN grade 3
3 RD	77	NAD	None taken	CIS	CIN grade 3
4 WB	62	NAD	None taken	CIS	CIN grade 3 plus invasive squamous ca
5 JS	39	*Irregular vessels on* anterior lip	? invasive squamous ca	Suggestive of squamous ca	Nonkeratinizing squamous ca
6 FM	31	Irregular vessels	None taken	Suggestive of squamous ca	CIN grade 3 plus microinvasive squamous ca
7 CC	24	Irregular vessels on *posterior lip*	Probable ca ? differentiation	Suggestive of squamous ca	Nonkeratinizing squamous ca
8 LR	30	Discrete area ulceration	? invasive squamous ca	Suggestive of squamous ca	Microinvasive squamous ca
9 EB	67	Not examined	None taken	Suggestive of adenocarcinoma	*Adenocarcinoma of* endocervix
10 JB	78	Ulceration & irregular *vessels*	CIS & adenocarcinoma	Mixed pattern	Same as for colposcopic *biopsy no further surgery*
11 SW	74	Nodule in vault	None taken	Suggestive of adenocarcinoma	Metastatic adenocarcinoma in vaginal vault
12 EH	84	Irregular vessels	Normal squamous epithelium only	Mixed pattern	Not established *(no further* surgery)
13 JF	70	*Multiple areas with* irregular vessels	Normal squamous epithelium only	Suggestive of adenocarcinoma	Not established (no *communication from* referring hospital)

TZ = transformation zone
CIS = carcinoma *in situ*
CIN = cervical intraepithelial neoplasia Grade 1 = mild dysplasia Grade 2 = moderate dysplasia Grade 3 = severe dysplasia and carcinoma in situ
NAD = nothing abnormal discovered

rather darkly staining cell borders *Metaplastic cells* were smaller with relatively less cytoplasm in all cells in the group in the plane of section more dense cytoplasm and at high magnification quite prominent cell bridges columnar cells showed cytoplasmic vacuolation suggesting the presence of mucus

The ultrastructure of these cells showed features similar to those described by Dembitzer et al [2] Superficial squamous cells had small nuclei in relation to the cytoplasm with marked margination of the chromatin and scanty organelles Numerous fibrils within *the cytoplasm either showed a peripheral distribution* or were uniformly dispersed and the cell surfaces showed variable cytoplasmic processes Some processes *were very prominent and regular but others* were more irregular and still others were more or less flat Desmosomes were observed but they tended to be sparse and poorly formed in these fragments of superficial epithelium When tissue fragments of endocervical epithelium were seen they showed a full thickness of epithelium with an underlying basement membrane and some stroma This is illustrated in Figures 1A and 1B where in addition to surface columnar cells there are more deeply situated metaplastic cells showing desmosomal attachments *and tonofibrils within the cytoplasm* Columnar cells were characterized by an irregular nucleus situated at the base of the cell mucus droplets in the apical cytoplasm and surface microvilli These columnar cells on the whole were closely apposed to one another while metaplastic cells showed wider intercellular spaces with prominent microvilli Single columnar cells and metaplastic cells also occurred the former were sometimes round in outline but they retained the characteristically irregular nucleus with rather coarsely distributed chromatin together with mucus droplets

Abnormal Cell Groups

At the light level single dyskaryotic cells were occasionally seen in thick sections but their occurrence was too variable and unreliable to make it worth *while collecting material* from patients with only mild or moderate dysplasia Satisfactory material could be obtained however from patients with severe dysplasia and carcinoma *in situ* when groups and sheets of epithelium were exfoliated as well as from those patients with problematic groups of cells Fragments of abnormal epithelium in the thick sections all showed remarkably similar appearances the only noteworthy diagnostic features were the presence of wide intercellular spaces and obvious intercellular bridges in the squamous lesions and the tightly apposed cells of adenocarcinomas

These differences were enhanced at the ultra*structural level so that differentiation between squa*mous lesions and adenocarcinoma was usually possible and in most instances a distinction could be made between *in situ* and invasive squamous lesions

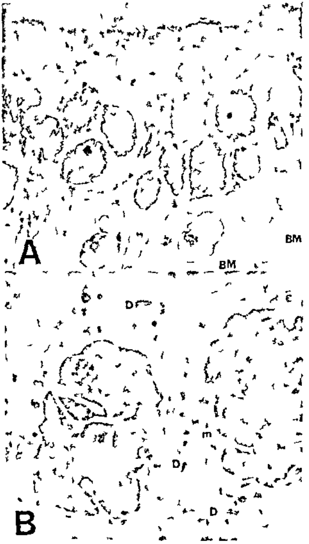

Figure 1
(A) Part of an endocervical fragment showing surface columnar cells with underlying metaplastic cells having wider intercellular spaces The basement membrane (BM) is just visible across [illegible] corner (uranyl acetate and lead citrate × [illegible]500) (B) Higher magnification of metaplastic cells showing irregular nuclei mitochondria (m) tonofibrils (t) and plentiful desmosomes (D) (uranyl acetate and lead citrate × 9 100)

Eight of the ten patients with thick groups of problematic cells in their cervical smears had ultrastructural features suggesting squamous differentiation Four of them were considered to have carcinoma *in situ* but in the other four a greater degree of abnormality suggested the possibility of invasive carcinoma One patient showed ultrastructural features of adenocarcinoma and one had a mixed pattern

Material from the vaginal vaults of two cases showed ultrastructural features suggesting adenocarcinoma and the third showed a mixed pattern with features of both glandular and squamous differentiation

Material with Ultrastructural Features of Carcinoma in Situ The fine structure of cell groups showing features of carcinoma *in situ* (Table I cases 1 to 4) was similar to that of the cells in previously examined biopsy material from patients with carcinoma *in situ* All cells in the fragment had relatively little cytoplasm in the plane of section with predominantly round or oval nuclei and although there was some irregularity of the nuclear membrane gross indentation of the nucleus was not a feature Chromatin distribution was moderately coarse and nucleoli were prominent (Figures 2A and 2B) Mitotic figures were demonstrated in some cases (Figure 2A) In the cytoplasm free ribosomes were numerous and there were occasional mitochondria but elements of endoplasmic reticulum although present were not a prominent feature and tonofibrils were scanty Desmosomes were present although in reduced numbers in comparison with normal parabasal cells and surface microvilli appeared to be increased

Subsequent histologic examination of material from these four patients confirmed the presence of carcinoma *in situ* in two and showed severe dysplasia in one If they are considered in terms of cervical intraepithelial neoplasia (CIN)[7,8] then all three could be designated CIN grade 3[1] Invasive squamous carcinoma was present in one case (case 4)

Material with Ultrastructural Features Suggestive of Squamous Carcinoma In four cases (cases 5 to 8) the fine structure of the cell groups was sufficiently different that the possibility of invasion was considered The cytoplasm was rather more abundant in all cells in the plane of section than in cells from carcinoma *in situ* and the cells were neither as orderly in arrangement nor as uniformly round The nuclei also showed more variability some were round or oval as in carcinoma *in situ* but large and multiple nucleoli

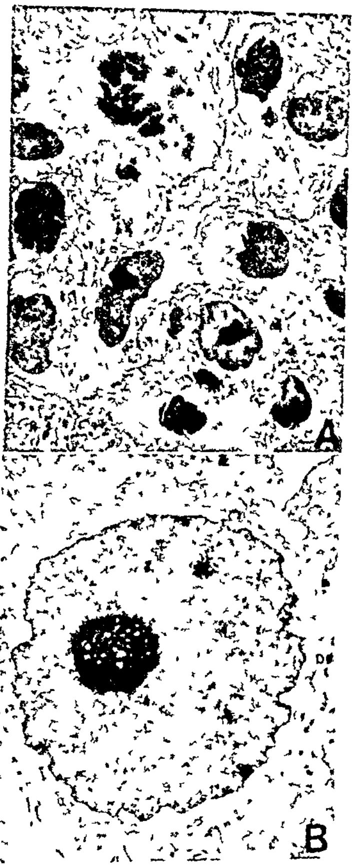

Figure 2

(A) Case 2 Electron micrograph of cell group showing features of *carcinoma in situ* with mitotic figure (uranyl acetate and lead citrate × 2 375) (B) Case 2 Part of cell group from patient with *carcinoma in situ* showing an individual cell with scanty cytoplasm prominent nucleolus and scanty desmosomes (D) (uranyl acetate and lead citrate × 11 37

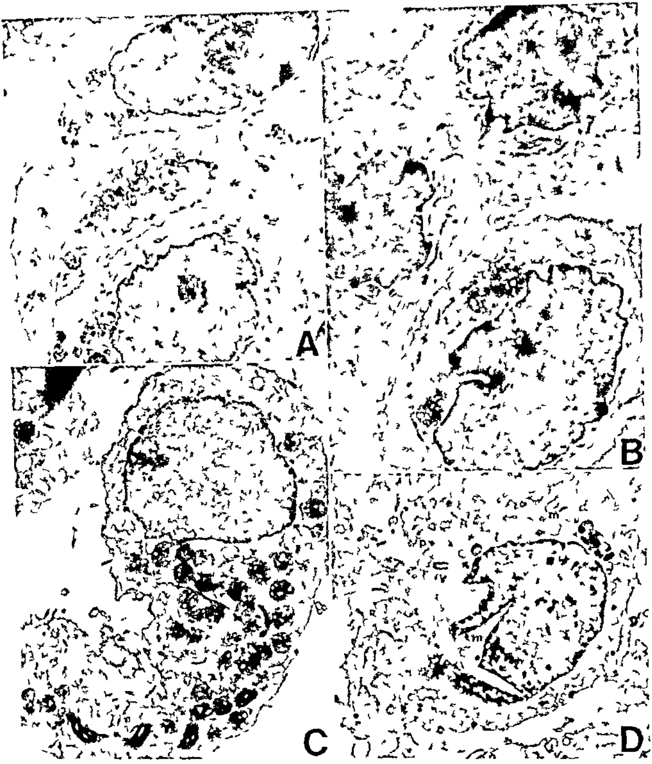

Figure 3
(A) Case 8 Electron micrograph of cell group from patient with microinvasive squamous carcinoma Cytoplasm is more abundant with more prominent organelles and tonofibrils (t) than in cells from carcinoma *in situ* (uranyl acetate and lead citrate × 5 625) (B) Case 8 Cells with irregular nuclei plentiful organelles and numerous tonofibrils (t) in material from patient with microinvasive squamous carcinoma (uranyl acetate and lead citrate × 11 375) (C) Case 8 *Single cell showing numerous multivesicular bodies and tonofibrils in the cytoplasmic tail* Material from patient with microinvasive squamous carcinoma (uranyl acetate and lead citrate × 11 375) (D) Case 5 Part of cell group from patient with invasive squamous carcinoma showing a cell with an irregular nucleus Polyribosomes mitochondria (m) and pinocytotic vesicles (p) are prominent features (uranyl acetate and lead citrate × 9 100)

were more frequent (Figure 3A) and others were much more irregular with more irregularly clumped chromatin (Figures 3B and 3D) Regardless of the nuclear characteristics elements of rough endoplasmic reticulum free ribosomes lysosomes and mitochondria were more prominent than in carcinoma *in situ* and the cell groups from three of the four cases showed numerous tonofibrils within the cytoplasm (Figures 3B and 3C) Desmosomes were probably more scanty than in groups from carcinoma *in situ* but they were by no means infrequent Case 8 also showed cells with numerous multivesicular bodies (Figures 3A 3B and 3C)

Invasive squamous carcinoma was histologically confirmed in these four patients it was microinvasive in cases 6 and 8 and deeply invasive in cases 5 and 7

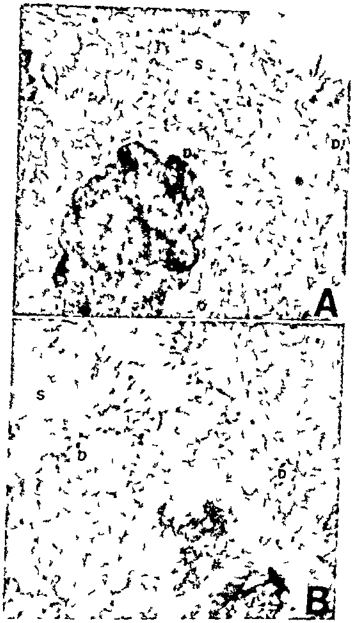

Figure 4
(A) Case 9 Part of cell group from patient with adenocarcinoma of the endocervix showing slight nuclear irregularity with chromatin clumping mucus droplets (S) and narrow intercellular spaces with desmosomes (D) (uranyl acetate and lead citrate × 9 100)
(B) Case 9 Another area of cell group from patient with adenocarcinoma of the endocervix showing mitotic figure narrow intercellular spaces with desmosomes (D) and mucus droplets (S) (uranyl acetate and lead citrate × 7 100)

Material with Ultrastructural Features of Adenocarcinoma The fine structure of cell groups collected from the cervix of case 9 suggested the presence of adenocarcinoma of the endocervix These cells were also irregularly arranged with nuclei showing coarsely clumped chromatin (Figure 4A) mitotic figures also were present (Figure 4B) However a columnar configuration could frequently be seen in cells surrounding glands or clefts and as in their normal counterparts they showed narrow intercellular spaces with scant microvilli numerous desmosomes and tight junctions near luminal borders Mitochondria lysosomes (with occasional autophagic vacuoles) free ribosomes dilated rough endoplasmic reticulum and Golgi zones were all conspicuous but the most obvious diagnostic feature of glandular origin was the presence of mucus droplets in some cells (Figures 4A and 4B) Subsequent endocervical curettage and hysterectomy confirmed the presence of invasive adenocarcinoma of the endocervix in this patient

The fine structure of the cell groups from two of the cases where material was collected from the vaginal vault (cases 11 and 13) was considered to be compatible with the presence of vault deposits of endometrial adenocarcinoma but their appearances were not identical

The cell fragments from case 11 were rather degenerate but the morphology was sufficiently clear to show villus lined clefts narrow intercellular spaces with interdigitating cell membranes numerous mitochondria and rather low nuclear-cytoplasmic ratios In one cell probable intracytoplasmic lumina contained flocculent material and were lined by microvilli (Figure 5A)

Case 13 showed much better cell preservation and again there were narrow intercellular spaces with junctional complexes numerous mitochondria and prominent endoplasmic reticulum but in this case nuclear irregularity was very marked and there were large nucleoli which had a loose coiled appearance with prominent nucleolonema (Figure 5B)

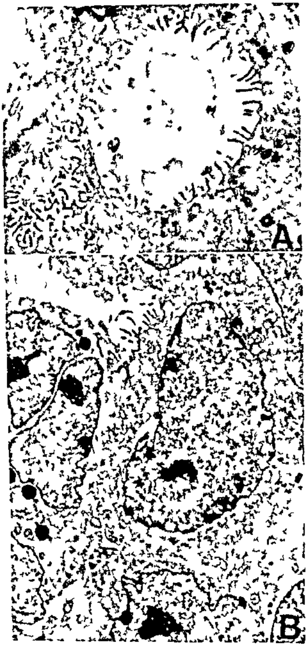

Figure 5
(A) Case 11 Cell showing intracytoplasmic lumina in material from patient with metastatic adenocarcinoma in vaginal vault (uranyl acetate and lead citrate × 8 875) (B) Case 13 Cell group from patient with probable vault deposit of endometrial adenocarcinoma showing cells with irregular nuclei and large nucleoli (uranyl acetate and lead citrate × 5 6_5)

Material with Ultrastructural Features of Both Squamous and Glandular Differentiation Material collected from the cervix of one patient (case 10) and the vault of another (case 12) showed features suggesting both squamous and glandular differentiation

The cervical smears from case 10 initially suggested the presence of a squamous carcinoma but over a five month period the appearances changed slightly so that features suggesting squamous differentiation (keratinization) disappeared and only abnormal cells with high nuclear-cytoplasmic ratios and hyperchromatic nuclei were present as single cells or cell groups Cellular samples for electron microscopy were collected at this time and the fine structure of these cell groups revealed several interesting features

The majority of the cell groups showed features of glandular differentiation with closely apposed cells prominent endoplasmic reticulum and mucus droplets (Figure 6A) Nuclei were slightly irregular but the chromatin was more evenly distributed than in the case of invasive adenocarcinoma of the endocervix described above (case 9) Numerous mitotic figures were present nucleoli were large and multiple in some cells Mitochondria were numerous and well preserved There were occasional large and complex residual bodies and one cell was ciliated (Figure 6B)

In addition to these cell groups with typical glandular morphology there were others which appeared to be squamous in type (Figure 6C) The cells of these groups were more elongated and the intercellular spaces were wider with microvilli Free ribosomes were more prominent and some of these cells had bundles of tonofibrils which supported their squamous differentiation

This patient had two cervical biopsies and both showed dysplasia or carcinoma *in situ* together with abnormal endocervical glands They showed a complex pattern with mitoses the appearances were compatible with either *in situ* or well-differentiated invasive adenocarcinoma of the endocervix

The cell groups from the vaginal vault of case 12 showed narrow intercellular spaces and cells with irregular nuclei dilated endoplasmic reticulum and mucus droplets characteristic of a glandular lesion In one area however cells of this type were seen to be attached to cells with no mucus droplets but with quite prominent tonofibrils The spaces between these two types of cells were wider than between the glandular cell types and microvilli were present

Unfortunately surgery has not been attempted in this patient and there is therefore no histologic confirmation of vault adenocarcinoma or of the presence of any squamoid features were more frequent (Figure 3A) and others were much more irregular with more irregularly clumped chromatin (Figures 3B and 3D) Regardless of the nuclear characteristics elements of rough endoplasmic reticulum free ribosomes lysosomes and mitochondria were more prominent than in carcinoma *in situ* and the cell groups from three of the four cases showed numerous tonofibrils within the cytoplasm (Figures 3B and 3C) Desmosomes were probably more scanty than in groups from carcinoma *in situ* but they were by no means infrequent Case 8 also showed cells with numerous multivesicular bodies (Figures 3A 3B and 3C)

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Material with Ultrastructural Features of Adeno carcinoma The fine structure of cell groups collected from the cervix of case 9 suggested the presence of adenocarcinoma of the endocervix These cells were also irregularly arranged with nuclei showing coarsely clumped chromatin (Figure 4A) mitotic figures also were present (Figure 4B) However a columnar configuration could frequently be seen in cells surrounding glands or clefts and as in their normal counterparts they showed narrow intercellular spaces with scant microvilli numerous desmosomes and tight junctions near luminal borders Mitochondria lysosomes (with occasional autophagic vacuoles) free ribosomes dilated rough endoplasmic reticulum and Golgi zones were all conspicuous but the most obvious diagnostic feature of glandular origin was the presence of mucus droplets in some cells (Figures 4A and 4B) Subsequent endocervical curettage and hysterectomy confirmed the presence of invasive adenocarcinoma of the endocervix in this patient

The fine structure of the cell groups from two of the cases where material was collected from the vaginal vault (cases 11 and 13) was considered to be compatible with the presence of vault deposits of endometrial adenocarcinoma but their appearances were not identical

The cell fragments from case 11 were rather degenerate but the morphology was sufficiently clear to show villus lined clefts narrow intercellular spaces with interdigitating cell membranes numerous mitochondria and rather low nuclear-cytoplasmic ratios In one cell probable intracytoplasmic lumina contained flocculent material and were lined by microvilli (Figure 5A)

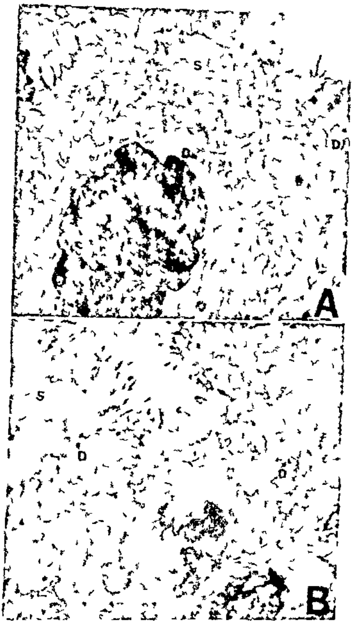

Figure 4

(A) Case 9 Part of cell group from patient with adenocarcinoma of the endocervix showing slight nuclear irregularity with chromatin clumping mucus droplets (S) and narrow intercellular spaces with desmosomes (D) (uranyl acetate and lead citrate × 9 100)
(B) Case 9 Another area of cell group from patient with adenocarcinoma of the endocervix showing mitotic figure narrow intercellular spaces with desmosomes (D) and mucus droplets (S) (uranyl acetate and lead citrate × 7 100)

Case 13 showed much better cell preservation and again there were narrow intercellular spaces with junctional complexes numerous mitochondria and prominent endoplasmic reticulum but in this case nuclear irregularity was very marked and there were large nucleoli which had a loose coiled appearance with prominent nucleolonema (Figure 5B)

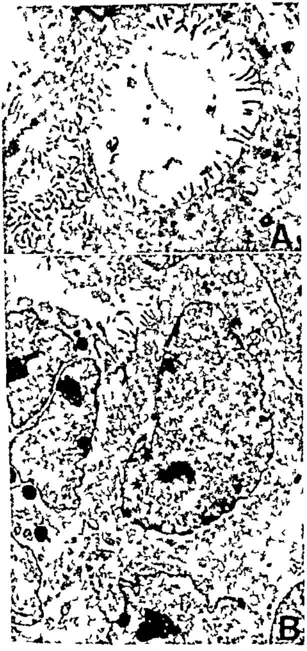

Figure 5
(A) Case 11 Cell showing intracytoplasmic lumina in material from patient with metastatic adenocarcinoma in vaginal vault (uranyl acetate and lead citrate × 8 875) (B) Case 13 Cell group from patient with probable vault deposit of endometrial adenocarcinoma showing cells with irregular nuclei and large nucleoli (uranyl acetate and lead citrate × 5 6 5)

Material with Ultrastructural Features of Both Squamous and Glandular Differentiation Material collected from the cervix of one patient (case 10) and the vault of another (case 12) showed features suggesting both squamous and glandular differentiation

The cervical smears from case 10 initially suggested the presence of a squamous carcinoma but over a five month period the appearances changed slightly so that features suggesting squamous differentiation (keratinization) disappeared and only abnormal cells with high nuclear-cytoplasmic ratios and hyperchromatic nuclei were present as single cells or cell groups Cellular samples for electron microscopy were collected at this time and the fine structure of these cell groups revealed several interesting features

The majority of the cell groups showed features of glandular differentiation with closely apposed cells prominent endoplasmic reticulum and mucus droplets (Figure 6A) Nuclei were slightly irregular but the chromatin was more evenly distributed than in the case of invasive adenocarcinoma of the endocervix described above (case 9) Numerous mitotic figures were present nucleoli were large and multiple in some cells Mitochondria were numerous and well preserved There were occasional large and complex residual bodies and one cell was ciliated (Figure 6B)

In addition to these cell groups with typical glandular morphology there were others which appeared to be squamous in type (Figure 6C) The cells of these groups were more elongated and the intercellular spaces were wider with microvilli Free ribosomes were more prominent and some of these cells had bundles of tonofibrils which supported their squamous differentiation

This patient had two cervical biopsies and both showed dysplasia or carcinoma *in situ* together with abnormal endocervical glands They showed a complex pattern with mitoses the appearances were compatible with either *in situ* or well-differentiated invasive adenocarcinoma of the endocervix

The cell groups from the vaginal vault of case 12 showed narrow intercellular spaces and cells with irregular nuclei dilated endoplasmic reticulum and mucus droplets characteristic of a glandular lesion *In one area however cells of this type were seen to be* attached to cells with no mucus droplets but with quite prominent tonofibrils The spaces between these two types of cells were wider than between the glandular cell types and microvilli were present

Unfortunately surgery has not been attempted in this patient and there is therefore no histologic confirmation of vault adenocarcinoma or of the presence of any squamoid features

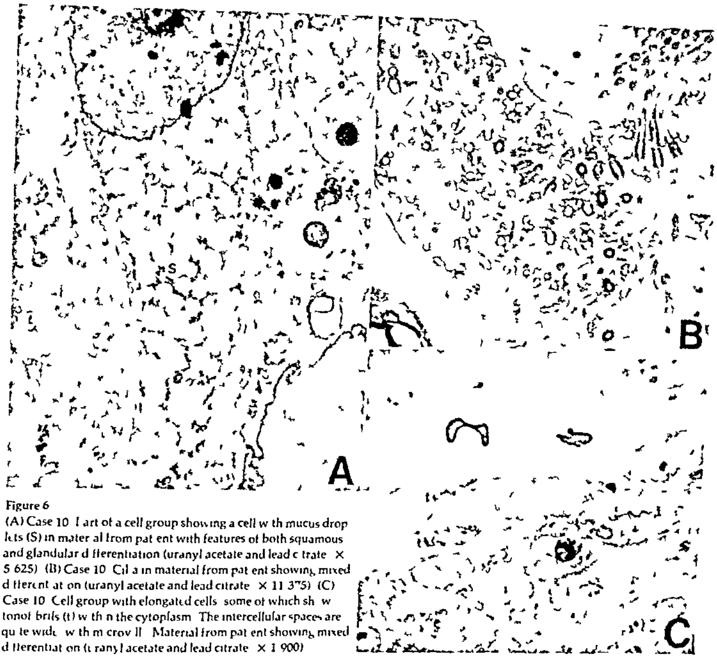

Figure 6
(A) Case 10 Part of a cell group showing a cell with mucus droplets (S) in material from patient with features of both squamous and glandular differentiation (uranyl acetate and lead citrate × 5 625) (B) Case 10 Cilia in material from patient showing mixed differentiation (uranyl acetate and lead citrate × 11 375) (C) Case 10 Cell group with elongated cells some of which show tonofibrils (t) within the cytoplasm The intercellular spaces are quite wide with microvilli Material from patient showing mixed differentiation (uranyl acetate and lead citrate × 1 900)

Discussion

The electron microscope has been used previously to study the morphology of both normal and abnormal exfoliated cells from the uterine cervix but the morphology of the abnormal cells has been described only briefly In 1960 Hanschke and Schulz[6] examined the fine structure of osmium fixed cells scraped from the vaginas and the cervices of 15 women five of whom had a cervical carcinoma *They claimed that it* was possible to differentiate between normal and malignant cells but that as at light level it was a combination of factors which enabled them to recognize the malignant cells Greider and Scarpelli in 1964[5] also examined osmium fixed scrapings from the surface of untreated cervical carcinomas and described slender tumor cells from a well-differentiated *squamous carcinoma Most of the cytoplasm con*tained fine unorientated tonofilaments desmosomes were not as highly developed as those in normal cells and only a few mitochondria and elements of the endoplasmic reticulum were present

In this investigation material was collected for examination of the fine structure of cells from patients who had had cervical smears which contained thick groups of cells with a high nuclear-cytoplasmic ratio and hyperchromatic nuclei similar groups were identified in plastic-embedded material They were found to be epithelial fragments in which the constituent cells retain their original attachments Squamous lesions both *in situ* and invasive were characterized by wide intercellular spaces and the presence of tonofibrils while fragments from adenocarcinomas showed narrow intercellular spaces with tight junctions near luminal borders prominent Golgi zones and rough endoplasmic reticulum

Material collected for electron microscopy was studied before the final histologic diagnosis was known and in four of the eight cases in which abnormal cells showed evidence of squamous differentiation additional features were present Pleomorphism was present with marked nuclear and nucleolar abnormality in some cells and there were prominent mitochondria ribosomes and elements of endoplasmic reticulum It was considered that these appearances probably reflected an invasive carcinoma and this was confirmed in all cases The features suggesting invasion were not found in a fifth case (case 4) and it is of importance to consider whether this was due to a sampling error in the material collected or whether it was an indication of the unreliability of ultrastructural appearances as an indication of invasion In this particular case a sampling error is possible because subsequent histologic examination of the cervix showed no abnormality of the ectocervix intraepithelial neoplasia and invasive carcinoma were present only in the endocervical canal This contrasts with the cases correctly diagnosed in which material was collected from areas of vascular irregularity (cases 5 to 7) or from a small discrete ulcer (case 8) It may also be relevant that in three of the four cases there was little intraepithelial neoplasia (cases 5 7 and 8) It is of interest that two of the four cases showing pleomorphism at the ultrastructural level (but not with light microscopy) were found to have microinvasion only on a detailed examination of the cervix It is obvious that many more cases will need to be examined before the observations made in this small series can be confirmed

Examination of the fine structure has been particularly useful in distinguishing adenocarcinoma from squamous lesions when this has not been apparent in conventional cervical or vaginal smears In addition the presence of numerous mucus droplets in some cells indicated cases of endocervical as opposed to endometrial adenocarcinoma The presence of a single ciliated cell in material collected from case 10 is of interest It formed part of an abnormal fragment of columnar epithelium in a patient with an incomplete histologic examination Biopsies showed squamous carcinoma *in situ* with *in situ* or well-differentiated adenocarcinoma but colposcopic examination showed ulceration and irregular vessels because the woman was old and in poor health she was transferred for radiotherapy Ciliated cells have been reported in other adenocarcinomas of the female genital tract[1,3,9] and ovary[4] and although we have found no report of ciliated cells in endocervical adenocarcinoma it would not be surprising to find them in reduced numbers as is reported in cases of well differentiated adenocarcinoma of the endometrium[3]

With the increase in facilities for electron microscopy this technique has a practical application when patients who are known to have equivocal cervical smears are examined colposcopically Scrape material from specific areas can be collected as described in this paper or smears can be made which are fixed in glutaraldehyde so that groups of cells which are difficult to interpret can be removed from the slide and processed for transmission electron microscopy This application will be described in a later publication

Acknowledgments

The authors wish to thank Jean Mather for her invaluable assistance with the electron microscopy and the photography

References

1 Cummins PA Fo H Langley FA An electron microscopic study of the endometrioid adenocarcinoma of the ovary and a comparison of its fine structure with that of normal endometrium and of adenocarcinoma of the endometrium J Pathol 113 165 173 1974

2 Dembitzer HM Herz F Schreiber K Wolley RC Koss LG The fine structure of exfoliated cervical and vaginal cells Acta Cytol 20 243 248 1976

3 Ferenczy A Richart RM Scanning electron microscopy of hyperplastic and neoplastic endometria *In* Scanning Electron Microscopy 1973 Part III Proceedings of the Workshop on Scanning Electron Microscopy in Pathology Edited by O Johari Chicago IIT Research Institute 1973 pp 613-620

4 Gondos B Electron microscopic study of papillary serous tumours of the ovary Cancer 27 1455 1464 1971

5 Greider H Scarpell DG The application of electron microscopy to the study of exfoliated cells Acta Cytol 8 39-44 1964

6 Hanschke HJ Schulz H Elektronenmikroskopische befunde an zellen von vaginal und portioabstrichen Arch Gynaekol 19 393-411 1960

7 Koss LG Nomenclature of precancerous and early cancerous lesions of the uterine cervix Contemp Obstet Gynecol 1 1-8 1978

8 Richart RM Barron BA A follow-up study of patients with cervical dysplasia Am J Obstet Gynecol 105 386-393 1969

9 Thrasher TV Richart RM An ultrastructural comparison of endometrial adenocarcinoma and normal endometrium Cancer 29 1713 1723 1972

The Cytology of Cerebral Neuroblastoma

Angelo Gandolfi, M D

A case is presented in which smears and touch imprints of a temporal tumor in a young adult male revealed fairly uniform round or polygonal cells with fine nuclear chromatin and scanty cytoplasm Occasional rosette formation was detected in most of the smears The cytology of these small tumor cells and their arrangement were useful in establishing the intraoperative diagnosis of cerebral neuroblastoma

The usefulness of cytologic methods in the rapid pathologic diagnosis of brain tumors has been stressed previously,[1-3] Smears and touch imprints were used in a recently observed case of cerebral neuroblastoma With this technique an accurate intraoperative diagnosis of this rare neoplasm became available to the neurosurgeon

Case Report

A 29 year-old white male was admitted to the Neurosurgical Service for evaluation of left homonymous hemianopsia transient left facial nerve paresis of central type left-sided hyperreflexia muscular weakness and Babinski s sign Computerized tomography showed a large and sharply defined right temporal mass which required an exploratory craniotomy

A soft granular pearly grey tumor well demarcated in places was found just beneath the cortex of the superior temporal gyrus with deep extension into the Sylvian fissure Fresh-cut surfaces were prepared from the gross specimens and several glass slides were touched to the tissue while several others were smeared They were immersed in an alcohol ether solution for one minute After fixation the slides were stained with routine hematoxylin and eosin stains for use with frozen sections

Results

The smears and touch imprints of the tumor were highly cellular and isomorphic (Figure 1) The tumor cells appeared small and round sometimes larger and polygonal with a wispy ill-defined cytoplasm These cells were arranged in irregular sheets or large clusters with a mosaic pattern showing areas of dense cellular packing True rosettes were easily detected in most of the specimen (Figures 2A to 2C) while incomplete poorly formed rosettes could be identified occasionally The nuclei in the rosette forming cells showed a tendency to be spindle shaped with the larger part of the nucleus at the periphery and the cytoplasm concentrated toward the center The nuclei were deeply stained round or slightly oval and fairly regular in size and shape They showed a sharply demarcated nuclear membrane and finely granular chromatin Sometimes one or two round and peripherally placed nucleoli could be seen Mitotic figures were seldom seen

Tissue section examination revealed a neuroblastoma with rare and sometimes incompletely defined rosettes (Figure 3)

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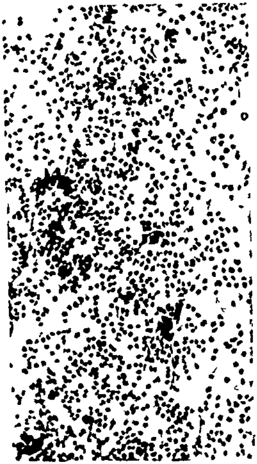

Figure 1
Smear from the tumor showing sheets of fairly uniform densely packed cells with round sometimes polygonal deeply stained nuclei and scanty ill-defined cytoplasm (hematoxylin and eosin × 50)

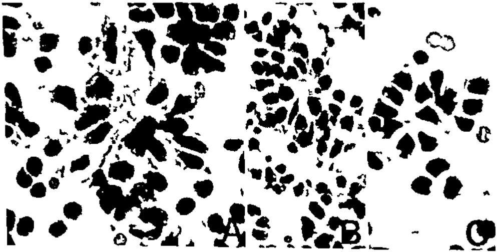

Figure
(A B C) Diagnostic rosettes in touch imprints of the tumor specimens The nuclei are peripherally placed and a streaming effect of the cytoplasm is visible (hematoxylin and eosin × 60 [A] × 1.5 [B] × 160 [C]

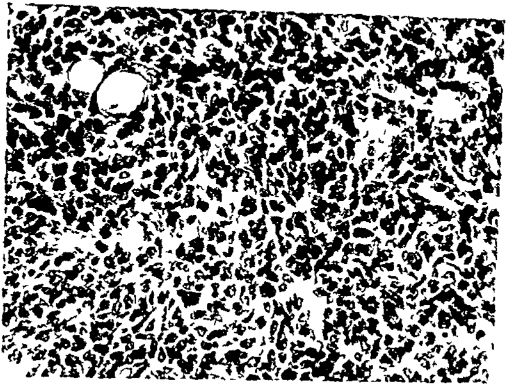

Figure 3
Histology of the tumor showing a highly cellular neoplasm without a definite cell arrangement Tumor cells are small with round or oval usually hyperchromic nuclei and ill-defined cytoplasm Nucleoli are sometimes evident A single rosette is visible in the upper right part of the figure (hematoxylin and eosin × 250)

Discussion

Cerebral neuroblastomas are rare highly cellular primitive neuroectodermal tumors which may be found anywhere in the hemispheres[4,6,8] They show a fairly high degree of malignancy and usually occur as rapidly growing neoplasms in children[3,6] Their histologic diagnosis is easily overlooked they are frequently reported as unclassifiable small cell malignancies because of their poor cytologic differentiation[3,8] *In the present case the correct interpretation* of smears and *imprints* was possible on the basis of typical cytologic morphology tumor cell arrangement and the presence of diagnostic true rosettes[1,2,9] These characteristic findings permitted excluding the cytologic diagnosis of astrocytoma[5,9] which is the most common tumor of the temporal lobe in young adults Furthermore the anatomic location of the neoplasm was not compatible with those of other tumors such as medulloblastoma retinoblastoma and pineoblastoma which exhibit strikingly similar cytomorphology to that of neuroblastoma[9]

It is noteworthy that the most useful diagnostic feature of this tumor true rosettes[1-8,9] was more easily detected in the cytologic than in the routine histologic preparations This report emphasizes the value of imprint and smear preparations in the daily practice of surgical neuropathology

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Cells of Nucleus Pulposus in Cerebrospinal Fluid

A Case Report

Gladwyn Leiman M B B Ch
Colin Klein M B B Ch
Ann V Berry M D F I A C

A case is presented in which inadvertent needling of the nucleus pulposus produced unusual cells in cerebrospinal fluid which caused diagnostic difficulty The case is documented to alert those involved in the diagnostic cytology of cerebrospinal fluid to yet another source of extraneous cellular material in fluid obtained by lumbar puncture

The normal path of the needle used to obtain cerebrospinal fluid (CSF) by lumbar puncture traverses skin subcutaneous adipose tissue and ligamentous tissue before entering the vertebral canal Misdirection of the needle or anatomic distortion of the lumbar spine may result in the puncture of the cartilaginous or even bony structures by the needle tip Cells of vascular cartilaginous hemopoietic or neural origin alien to normal CSF have been described as accidental findings when encountered in cytologic preparations[1] The purpose of this paper is to alert cytologists to another unusual source of cellular material in CSF obtained by lumbar puncture

Case History

A 50 year-old man was admitted to Baragwanath Hospital with a three week history of progressive weakness and burning pain starting in the feet and ascending to the pelvis His only other complaint was of a sore throat which began seven days before admission Significant clinical findings were pallor pyrexia and severe pharyngitis Lower limb weakness was confirmed with brisk reflexes and increased tone detected bilaterally Decreased sensation was present from T10 downwards X ray examination showed numerous lytic lesions in the vertebrae

Investigations on admission showed a hemoglobin of 7 2 gm% and a white cell count of 10 800 per cu mm Bone marrow aspiration demonstrated prominent plasma cells suggestive of myeloma Protein electrophoresis showed a confirmatory diagnostic pattern with a total protein of 12 20 gm/dl constituted as follows albumin 2 35 gm/dl globulin 9 85 gm/dl $\alpha 1$ 0 53 gm/dl $\alpha 2$ 1 06 gm/dl $\beta 2$ 0 90 gm/dl γ 6 36 gm/dl Immunoglobulin levels were IgG 8 000 mg/dl IgA 24 mg/dl IgM 80 mg/dl

In view of the paraplegia a myelogram was performed Marked difficulty was experience in entering the subarachnoid space On the first attempt needle resistance was met and on withdrawal of the needle a core of semisolid tissue was found in the needle bore It was submitted for cytologic examination The second attempt at myelography was successful and demonstrated a total block from T8 to T10

The CSF was macroscopically clear with a protein content of 2 250 mg% and glucose of 79 mg% The lumbar puncture was repeated the following day confirming the high protein content initially detected This specimen however was acellular

Cytologic Findings

The specimen was prepared with a Gelman filter and

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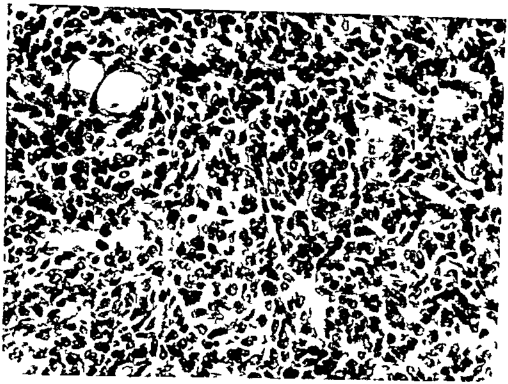

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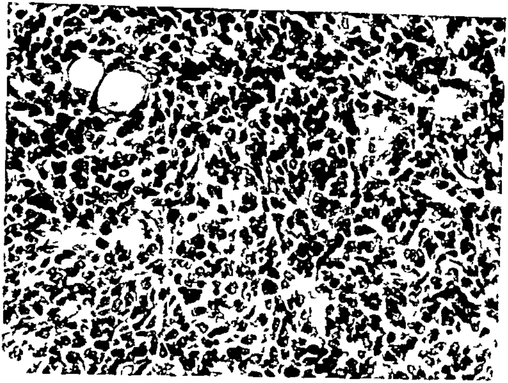

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Investigations on admission showed a hemoglobin of 7 2 gm% and a white cell count of 10 800 per cu mm Bone marrow aspiration demonstrated prominent plasma cells suggestive of myeloma Protein electrophoresis showed a confirmatory diagnostic pattern with a total protein of 12.20 gm/dl constituted as follows albumin 2 35 gm/dl globulin 9 85 gm/dl $\alpha 1$ 0 53 gm/dl $\alpha 2$ 1 06 gm/dl $\beta 2$ 0 90 gm/dl γ 6 36 gm/dl Immunoglobulin levels were IgG 8 000 mg/dl IgA 24 mg/dl IgM 1 80 mg/dl

In view of the paraplegia a myelogram was performed Marked difficulty was experienced in entering the subarachnoid space On the first attempt needle resistance was met and on withdrawal of the needle a core of [illegible] tissue was found in the needle bore It was submitted for cytologic examination The second attempt at myelography was successful and demonstrated a total block from T8 to T10

The CSF was macroscopically clear with a protein content of 2 250 mg% and glucose of 77 mg% The lumbar puncture was repeated the following [illegible] the high protein content [illegible] detected This specimen however was acellular

Cytologic Findings

The specimen was prepared with a Gelman filter and

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Cytosieve (Gelman Instrument Company Ann Arbor Michigan) of pore size 5µm and stained by the Papanicolaou method The fluid contained clusters and small fragments of two to ten cells Individual cells had an apparent eccentric nucleus and clear cytoplasm giving an initial impression of adenocarcinoma (Figure 1) However closer inspection showed that the cell walls were in fact fine but rigid capsules and that the true cells had retracted to the periphery of the capsules Each cell was small and bipolar containing a dense hyperchromatic round to-oval nucleus and well-defined cyanophilic cytoplasm

Neither the nature nor the source of these cells could be identified Diagnoses such as chordoma and *metastatic adenocarcinoma* were considered but discarded The possibility of derivation from bone or cartilage considering the difficult lumbar puncture was discussed but the cell morphology did not conform to documented examples of chondrocytes or osteocytes in fluid specimens[2] The only definite information which could be given to the clinicians at that stage was that the cells were not myeloma cells

In view of the above findings the patient underwent surgery and a thoracic laminectomy involving T8 to T10 was performed An extradural tumor was removed and histology showed the presence of myeloma (Figure 2) The patient received radiotherapy to the thoracic spine as well as chemotherapy

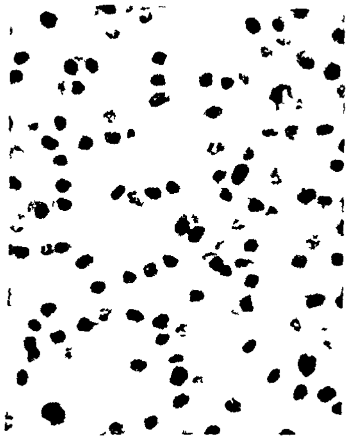

Figure 2
Section of extradural tumor showing malignant plasma cells *(hematoxylin and eosin × 400)*

Figure 3

Deliberation as to the nature and origin of the unexplained cytologic findings continued and the nucleus pulposus was ultimately suggested as a possibility Sections of a nucleus pulposus in the second lumbar interspace obtained at necropsy from a male cadaver of the same age as the patient showed the cell groupings illustrated in Figure 3 In that figure the rigid capsules are better seen and the small retracted bipolar cells appear to be exact replicas of those in the fluid specimen The enigma was explained

Discussion

The nucleus pulposus is a semisolid gelatinous mass derived from notochord It is composed of a faintly metachromatic matrix consisting of extremely delicate fibrous material With advancing age the nucleus pulposus undergoes progressive replacement by collagenous fibers and cartilage like cells so ultimately it may resemble fibrocartilage [1]

Inadvertent needling of the nucleus pulposus during misdirected lumbar puncture or myelography may yield cells in CSF which will create a diagnostic dilemma It is hoped that this paper will aid other cytologists confronted with this problem

References

1 Greep RD Histology Second edition London McGraw Hill 1966 p 15

2 Kolmel HW Atlas of Cerebrospinal Fluid Cells Second edition Berlin Springer Verlag 1977 pp 16 17

Metastatic Malignant Fibrous Histiocytoma in Lung Examined by Fine Needle Aspiration

Case Report and Literature Review

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Malignant fibrous histiocytoma appears to be emerging as one of the most common soft tissue sarcomas of late adult life Cytologic features of a metastatic malignant fibrous histiocytoma in the lung examined by fine needle aspiration are reported Essentially four cell types in various proportions were observed spindled cells pleomorphic giant cells undifferentiated mesenchymal type cells and rare signet ring lipoblast like cells There also were several huge hyperchromatic and often bizarre naked nuclei

Malignant fibrous histiocytoma (MFH) has recently been cited as the most common soft tissue sarcoma of late adult life [6] When the malignant potential of fibrous xanthoma was described by O Brien and Stout in 1964 it was assumed that the histiocyte had the dual ability of phagocytosis and of connective tissue fiber formation [4] While tissue cultures of tumor explants have supported the theory that the tissue histiocyte can act as a facultative fibroblast [3] it has also been suggested that MFH may arise directly from primitive mesenchymal cells which remain in adult tissues and differentiate under certain stimuli to produce specific neoplasms [2] Furthermore it is known that cells other than histiocytic (Schwann cells synovial cells or mesothelial cells) may dedifferentiate and act as facultative fibroblasts [3] Whether the origin of the neoplasm is histiocytic or mesenchymal or from other dedifferentiated cell types MFH is regarded at present as a primitive and pleomorphic sarcoma showing partial fibroblastic and partial histiocytic differentiation [6]

The histologic pattern of MFH is one of variability and these neoplasms have been named according to the predominant histologic component While soft tissue sarcomas are almost always made up of a conglomerate of a particular type of *neoplastic cell* MFH is composed mostly of fibroblast like and histiocyte-like cells in various proportions The classic growth pattern is that of fibroblastic and histiocytic cells arranged in a storiform pattern accompanied by pleomorphic giant cells and inflammatory cells Transition from areas with a highly organized storiform pattern to less well differentiated areas with a pleomorphic appearance is frequently seen in MFH [6]

Variants of MFH include the so-called pleomorphic fibrosarcoma malignant histiocytoma myxoid MFH malignant giant cell tumor of soft parts and malignant xanthogranuloma The myxoid variant of MFH has recently been described by Weiss and Enzinger [7] It is characterized by widely spaced spindled and pleomorphic cells embedded in a matrix of acid mucopolysaccharides The cellular areas are indistinguishable from those of the typical pleomorphic MFH Although myxoid change is commonly seen in MFH classification of a neoplasm as a myxoid variant should be restricted to those neoplasms whose overall appearance is at least 25% myxoid This variant appears to have a more favorable prognosis [7]

MFH occurs principally on the lower limbs the thigh is the most common site The peak incidence of this neoplasm is in the sixth and seventh decades It is found about twice as often in men as in women and

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occurs in white-skinned persons in over 90% of all cases [6] When the neoplasm is located on a limb the most common symptom is that of a painless enlarging mass usually of less than six months duration

Several factors influence the prognosis of MFH They include size and depth of the neoplasm degree of myxoid change and type of accompanying inflammation [7] The rate of metastasis is 42% and occurs most often to the lung (82%) [6] In one study the rate of metastasis was inversely proportional to the degree of myxoid change and those neoplasms accompanied by an acute inflammatory reaction had a slightly better prognosis than those accompanied by a chronic inflammatory reaction [7] Metastasis usually occurred about three years after the initial excision and the metastatic lesion was often more cellular than the primary neoplasm [7] In a study of 200 patients with MFH a significant number of patients developed local recurrence or meta tasis after two years and the two year survival rate was 60% [6]

Case Report

A 75 year-old man was admitted to our hospital in February 1979 with the chief complaints of hemoptysis and weakness of about two weeks duration In January 1978 a soft tissue mass had been excised from his left thigh at another hospital Although this lesion was initially thought to be a liposarcoma on review of the histologic sections it proved to be an MFH (Figures 1A and 1B) Excision of the neoplasm was followed by x ray therapy to the operative site The patient remained well and asymptomatic for about one year after treatment until the onset of his recent complaints

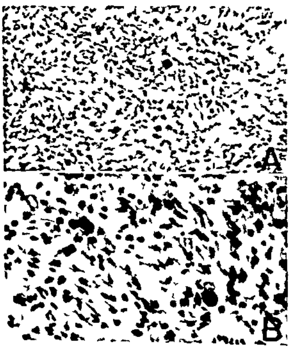


Figure 1
Histologic pattern of the MFH removed from the left thigh (A) Area showing distinct storiform pattern characteristic of MFH (hematoxylin and eosin × 100) (B) High-power view to illustrate fibrohistiocytic features and giant cells (hematoxylin and eosin × 250)

Physical examination showed no evidence of local recurrence of the neoplasm in the thigh X ray films of the chest revealed a 6×10-cm mass in the right lung and a right pleural effusion which increased in amount during the next two weeks causing a left mediastinal shift Tomography showed no narrowing of bronchi Radiologic examination of the skeleton brain liver and spleen did not reveal any neoplasm

Material was aspirated from the pulmonary mass through an 18-gauge needle under direct fluoroscopic control Repetitive turning of the needle and application of suction were employed to obtain cellular material in the manner popularized by Dahlgren and Nordenstrom [1] About 5 ml of yellowish glairy mucoid fluid was aspirated with a few small tissue fragments Smears and cell blocks were prepared and stained by the Papanicolaou method and with hematoxylin and eosin (H&E) In addition several smears and sections of the cell blocks were stained with the periodic acid Schiff (PAS) technique without diastase digestion with the Prussian blue method for iron and with Sudan IV for lipids All smears were fixed immediately in 95% ethanol except for a few which were air dried in preparation for the fat stain

Cytologic Findings

The smears were moderately cellular with a mucoid background whose staining reaction was blue with the Papanicolaou stain and pink with H&E Malignant neoplastic cells occurred singly as well as in loosely cohesive clusters with the single cells predominating

The most commonly observed type of cell was fusiform and contained one or two plump round to oval centrally located nuclei (Figure 2) The nuclear chromatin was characteristically finely granular slightly increased in amount and evenly distributed Distinct nucleoli were not seen and there was little if any variation in nuclear size The cytoplasmic staining reaction was a delicate pink with H&E Occa

Figure 2
Spindle-shaped fibroblast like cell. Note cytoplasmic tails and fine granular quality of the chromatin (hematoxylin and eosin × 250)

Figure 3
Multinucleated pleomorphic giant cell (hematoxylin and eosin × 250)

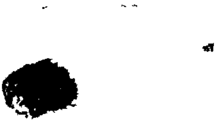


Figure 4
Mesenchymal cell. Note the lack of *cellular differentiation as evidenced by the scant* amount of cytoplasm (hematoxylin and eosin × 400)

sionally minute particles were seen in the cytoplasm of these cells, indicating their phagocytic capability. We interpreted these cells as representative of the fibroblast like cells of MFH. Such cells with ingested *particles were considered to be transitional forms intermediate between fibroblasts and histiocytes.*

Another commonly observed *malignant cell occurring singly was the pleomorphic giant cell* (Figure 3). Its cytoplasm was abundant, foamy or granular and pink with *H&E. The cell borders were irregular and ill-defined. Intracytoplasmic inclusions were sometimes seen. These cells contained multiple, centrally* located hyperchromatic *nuclei with sharply irregular* and angular borders. Chromatin was coarsely granular and irregularly distributed. We considered *these cells to represent the characteristic pleomorphic* giant cells of MFH.

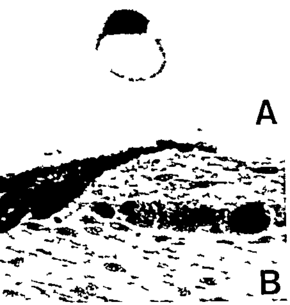
A *third type of malignant cell,* which was relatively *uncommon, occurred singly and was small and un*differentiated (Figure 4). It was characterized by an eccentric nucleus and very scant cytoplasm, which *was eosinophilic with the H&E stain. Its nucleus was* markedly hyperchromatic and irregularly angular and contained coarse, irregularly distributed clumps of chromatin. We interpreted these cells as being undifferentiated mesenchymal cells since no morphologic *differentiation could be detected in them.*

A fourth cell type observed with considerable frequency in the smears was the size of a mononuclear histiocyte. Its nucleus was small and oval and was pushed to the periphery of the cell by a single large intracytoplasmic vacuole. This nuclear displacement gave the cell a "signet ring" appearance (Figure 5A). Also present were other cells of the same size, which had finely vacuolated cytoplasm and single, eccentric, bean-shaped nuclei and which were histiocytic in appearance. When stained with Sudan IV, small cytoplasmic particles of fat positive material were present (Figure 5B). In none of the "signet ring" cells did we find the large cytoplasmic vacuole to stain as a single fat positive inclusion, as one would expect to see in a lipoblast.

In general, cell clusters were not encountered as commonly as were single cells. When present, they consisted of loosely cohesive cells of the fibroblastic type, with fibrillar cytoplasm (Figure 6).

Also noted were huge, irregularly shaped nuclei devoid of cytoplasm (Figures 7A and 7B). They were hyperchromatic, possessing a coarsely granular chromatin which displayed a reticular pattern. Some of the naked nuclei contained large irregular nucleoli which were often multiple. *Nucleoli were not noted in any of the other types of cells described above.*

The smears contained a few inflammatory cells, mostly lymphocytes. Plasma cells and eosinophilic leukocytes were not present, and neutrophilic leuko



gure 5
Signet ring cell (A) Nucleus pushed to the periphery of cell by a ngle large cytoplasmic vacuole (Papanicolaou stain × 400) (B) mall particles of fat positive material located intracellularly as ell as extracellularly are evidence of the phagocytic property of his cell type (Sudan IV stain × 400)

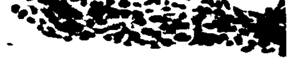


Figure 6
Fibroblast-like cells in a loosely cohesive cluster Note fibrillar nature of the cytoplasm and the round to oval nuclei showing little variation in shape and size (hematoxylin and eosin × 250)

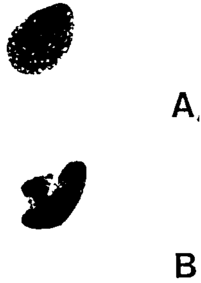


Figure 7
Pleomorphic naked nuclei (A) The reticular pattern of the chromatin is clearly seen (Papanicolaou stain × 250) (B) Note angular shape hyperchromasia and prominent nucleoli (Papanicolaou stain × 250)

cytes were rarely seen The cells stained negatively with the Prussian blue reaction and with the PAS reaction without diastase digestion

Discussion

MFH of soft parts is a neoplasm that is becoming recognized with increasing frequency as indicated by the numerous reports in the recent literature To be considered in the differential diagnosis of MFH are pleomorphic liposarcoma fibrosarcoma pleomorphic rhabdomyosarcoma pleomorphic carcinoma and Hodgkin s disease

Differentiating between MFH and pleomorphic liposarcoma may be difficult and the occasional neoplasm will demonstrate histologic features of both these neoplasms Liposarcoma may contain large multinucleated malignant cells in addition to the characteristic lipoblasts Cells can occur in small loose clusters as well as singly In an analysis of 200 cases of MFH [6] a lipid stain showed in most cases the presence of small fat droplets in foamy histiocyte like cells as well as in fibroblast like cells and giant cells In MFH the lipid was more finely dispersed or irregularly distributed and did not tend to displace the nucleus as in lipoblasts Lipoblast like cells with coarsely vacuolated cytoplasm and indented nuclei have been described in myxoid areas of MFH [7] but special stains have shown that these cells contain mucopolysaccharides instead of fat Liposarcoma tends to have a more cellular background in contrast to the myxoid background of MFH [7]

Fibrosarcoma is a neoplasm of younger adults occurring most commonly on the lower limbs The loosely cohesive clusters of spindled cells observed in MFH bear a strong resemblance to those seen in fibrosarcoma The cells in these clusters represent the malignant fibroblastic component common to both neoplasms [3] The characteristic cell type in fibrosarcoma is a fairly uniform plump spindled cell or a medium-sized round to oval cell Multinucleated giant cells are very uncommon in this neoplasm [3]

Pleomorphic rhabdomyosarcoma is a relatively rare tumor of adults, occurring more commonly in the fifth and sixth decades of life The major cell component in this neoplasm is a bizarre elongated cell with tadpole strap and racket forms and no tendency toward cellular cohesion Although cross striations are not often seen numerous long filaments are commonly recognized in the cytoplasm of the giant tumor cells [3]

Pleomorphic carcinoma can usually be distinguished by finding epithelial clusters and also by a careful search for mucin or glycogen which may be present in some of the cells

In Hodgkin s disease the cellular pattern is pleomorphic and the cells occur singly rather than in clusters The diagnosis can be made only when Reed-Sternberg cells are found

Conclusion

MFH should be considered in the differential diagnosis of pleomorphic sarcomas of soft parts both primary and metastatic The cytologic diagnosis of this neoplasm is based on the presence of spindled fibroblast like cells and rounded histiocyte like cells along with pleomorphic giant cells Lipid and phagocytosed particles in the malignant cells along with the absence of characteristic lipoblasts are helpful diagnostic features

References

1 Dahlgren S Nordenstrom B Transthoracic Needle Biopsy *Chicago Year Book Medical Publishers 1966 pp 29-41*

2 Fu YS Gabbiani G Kaye GI Lattes R Malignant soft tissue tumors of probable histiocytic origin (malignant fibrous histiocytoma) General consideration and electron microscopic and tissue culture studies Cancer 35 176-198 1975

3 Hajdu S Hajdu EO Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors Philadelphia WB Saunders 1976 pp 213 253

4 O'Brien J Stout AP Malignant fibrous xanthomas Cancer 17 1445-1455 1964

5 *Ozzello L Stout AP Murray MR Cultural characteristics of* malignant histiocytomas and fibrous xanthomas Cancer 16 331 344 1963

6 Weiss SW Enzinger FM Malignant fibrous histiocytoma An analysis of 200 cases Cancer 41 2250-2261 1978

7 Weiss SW Enzinger FM Myxoid variant of malignant fibrous histiocytoma Cancer 39 1672 1685 1977

Renal Oncocytoma

Preoperative Diagnosis by Aspiration Biopsy

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The cytologic histologic and ultrastructural features of an oncocytoma of the right kidney with the primary diagnosis by thin needle aspiration biopsy are presented These uncommon tumors are generally considered to be of renal tubular origin and are thought to be benign

In the case reported below two separate tumor masses were present within the diseased kidney one of them extended to the perirenal adipose tissue and was accompanied by satellite tumor nodules This presentation of oncocytoma associated with focal cellular atypia suggests that malignant behavior of at least some of these tumors may be anticipated

Fine needle aspiration biopsies guided by ultrasound television fluoroscopy or computerized scanning tomography (CAT scanning) are increasingly being used as a prompt minimally invasive method in the evaluation of tumors and tumorlike conditions of intrathoracic and intraabdominal organs An excellent review of this subject may be found in a recent publication by Zajicek [13] In experienced hands the advantages of this method of diagnosis are evident it may obviate the need for surgical exploration in some cases or alert the surgeon to the nature of the disease to be attacked by surgical means

We report below the first case of renal oncocytoma correctly identified on aspiration biopsy and subsequently studied by other means

Case Report

A 76 year-old Israeli man who spoke only Hebrew and was a poor historian was admitted to Montefiore Hospital and Medical Center in May 1978 because of a cyst in the kidney diagnosed on routine examination for hypertension in another hospital The only important episode of the past history was an uneventful transurethral resection of the prostate The patient was otherwise asymptomatic and the physical examination was negative except for an enlarged prostate and a blood pressure of 180/100 mm Hg He was receiving diuretics The laboratory data were within normal limits

Retrograde pyelogram revealed a mass impinging on the collecting system of the right kidney An angiogram was attempted but the patient became hypotensive and the procedure could not be completed On CAT scanning a solid mass in the upper right pole of the kidney was observed The mass appeared to be confined to the perirenal space and was interpreted as probably representing a renal carcinoma Nephrosonography disclosed a bulbous solid mass in the upper pole of the right kidney In the left kidney two presumably benign cysts one 6 cm in diameter at the upper pole and the other 2 cm in diameter at the lower pole were seen Aspiration smears were obtained under sonographic guidance from several areas of the mass in the right kidney The smears were immediately fixed in 95% ethyl alcohol and stained with Papanicolaou stain

Cytologic Observations

The smears contained numerous clusters of renal tubular cells characterized by abundant granular cytoplasm with frayed borders and monotonous small dark nuclei (Figure 1) In addition the smears

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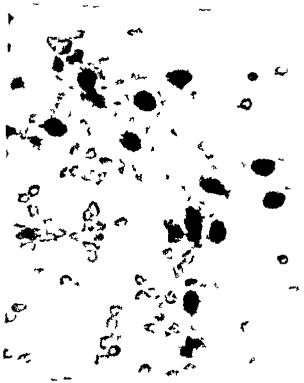
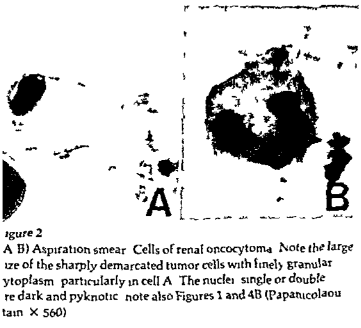


Figure 1
Aspiration smear Cluster of normal cells of renal tubular origin Note the granular generally frayed poorly demarcated cytoplasm and dark nuclei of monotonous size (Papanicolaou stain × 560)



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A B) Aspiration smear Cells of renal oncocytoma Note the large ze of the sharply demarcated tumor cells with finely granular ytoplasm particularly in cell A The nuclei single or double re dark and pyknotic note also Figures 1 and 4B (Papanicolaou tain × 560)

ontained a small number of much larger tumor cells ingly and in small clusters (Figure 2) The cells had bundant well-demarcated eosinophilic granular ytoplasm with smoothly outlined single or multi-le spherical or ovoid large nuclei Most of the nu-lei were hyperchromatic with marked chromatin lumping suggestive of pyknosis Numerous frag ents of renal capillaries blood and some necrotic aterial were also observed The paraffin-embedded residual material (cell block) contained isolated normal glomeruli (Figure 3) and cell clusters of renal tubular epithelial origin admixed with the larger tumor cells with glandular cytoplasm The large cells were interpreted as oncocytes and so reported

A right nephrectomy was performed The patient s recovery was uneventful and he has remained free of disease for one year

Gross Pathology Findings

The specimen measured 21×10×7 cm and contained a kidney (11×6×5 cm) surrounded by perirenal adipose tissue On section two separate tumor masses of a similar brown color were observed in the kidney One located in the lower pole measured 5×4×3 cm The other measuring 6×5×4 cm was located at the upper pole of the kidney This tumor extended directly into the perirenal adipose tissue where it produced a lobulated mass measuring 6×3×1 5 cm Two small satellite tumor nodules separated from the main tumor mass were also noted in the perirenal fat Renal vessels and the ureter were free of tumor Multiple thin walled benign cysts from 0 5 to 2 5 cm in diameter containing yellowish clear fluid were present throughout the entire kidney All of the tumor nodules and the adjacent kidney were extensively sampled by tissue blocks Fresh tumor tissue was fixed in 2 5% buffered glutaraldehyde for electron microscopic examination

Histologic Findings

The tumors were composed throughout of tubular structures of varying size made up of monotonous large cells with ample granular eosinophilic cytoplasm (Figure 4A) Most of the nuclei were vesicular round to oval and of even sizes some cells had double nuclei Focal nuclear atypia pleomorphism and nuclear pyknosis were observed (Figure 4B) Mitotic figures were not seen The satellite tumor nodules from the perirenal fat had an essentially similar histologic configuration None of the sections showed any similarity to the classical forms of renal carcinoma The diagnosis of renal oncocytoma with extension to the perirenal fat and probable metastases was established

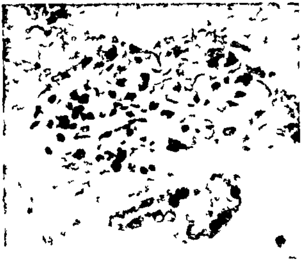


Figure 3
Cell block of aspirated material Normal renal glomerulus with attached fragment of renal convoluted tubule (hematoxylin and eosin × 350)

Electron Microscopic Findings

Ultrastructural studies showed the presence of large epithelial tumor cells with the cytoplasm crowded by huge numbers of enlarged round or oval mitochondria obscuring other cytoplasmic organelles (Figure 5) These findings were consistent with cells of the oncocytic type [11]

Discussion

The concept of oncocytes as a distinct type of cells of epithelial derivation was first proposed by Hamperl in 1931 in reference to salivary glands [5,7] The term *onkocytoma* was introduced by Jaffe again in refer

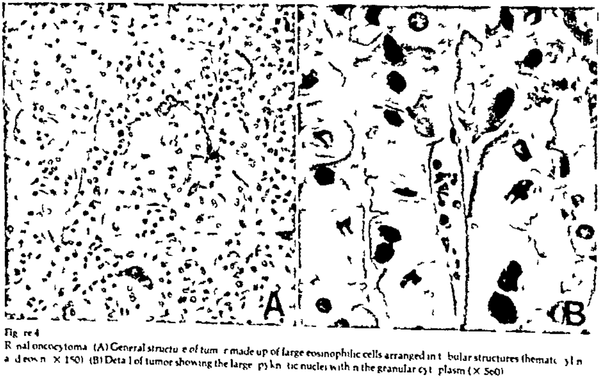
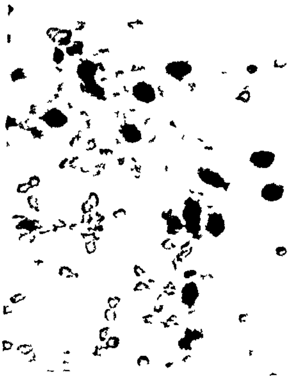


Figure 4
Renal oncocytoma (A) General structure of tumor made up of large eosinophilic cells arranged in tubular structures (hematoxylin and eosin × 150) (B) Detail of tumor showing the large pyknotic nuclei within the granular cytoplasm (× 560)



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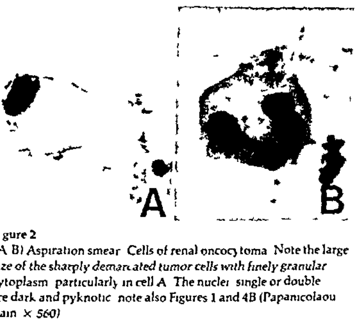

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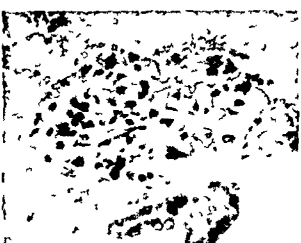

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Discussion

The concept of oncocytes as a distinct type of cells o epithelial derivation was first proposed by Hamper in 1931 in reference to salivary glands [5,7] The *term onkocytoma* was introduced by Jaffe again in refer

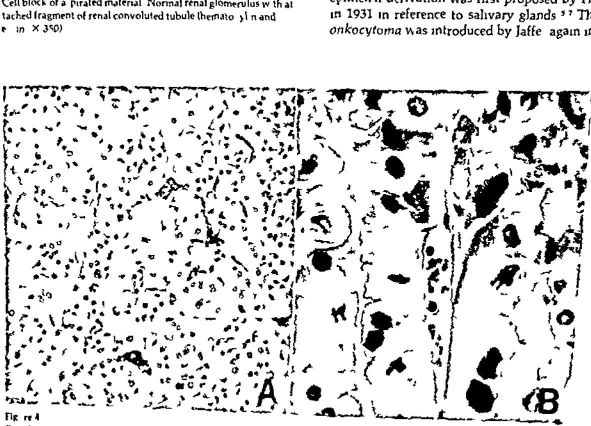

Figure 4
Renal oncocytoma (A) General structure of tumor made up of large eosinophilic cells arranged in tubular structures (hematoxylin and eosin × 150) (B) Detail of tumor showing the large pyknotic nuclei within the granular cytoplasm (× 560)

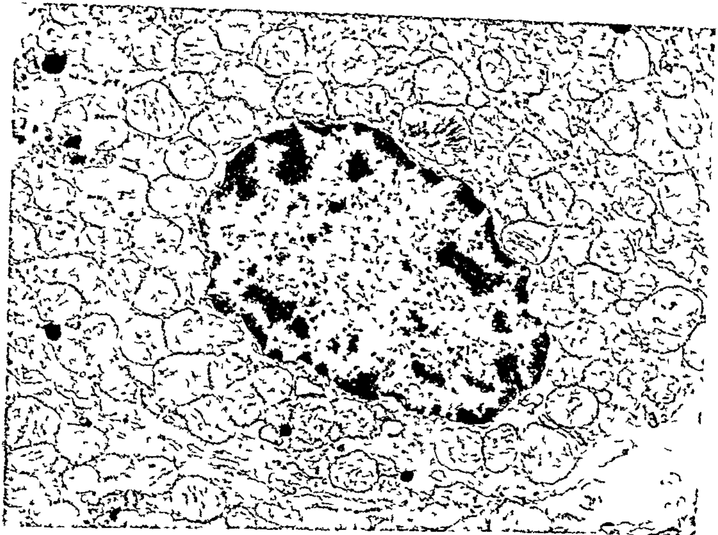

Figure 5
Electron micrograph of a tumor cell showing the characteristic cytoplasm crammed with mitochondria (approx × 8 000)

ence to a tumor of salivary glands otherwise known as an adenolymphoma or 'Warthin s tumor '[9] Subsequently these very characteristic large cells with granular eosinophilic cytoplasm and the tumors derived therefrom have been described in several organs notably the parotid [3] the thyroid [8] the parathyroid[6, 11] and the kidney [6, 10] While the majority of oncocytomas are considered benign their behavior cannot always be ascertained Thus oncocytomas of the thyroid (improperly referred to as 'Hurthle cell tumors) are fully capable of metastases as first documented on a large series of patients by Frazell and Duffy [4]

Klein and Valensi[10] recently reported on 13 oncocytic tumors of the kidney and emphasized their benign behavior These authors stressed that all of the tumors were single and confined to renal parenchyma without evidence of invasion of perirenal fat Hamperl [6] on the other hand recorded and illustrated two cases of malignant renal oncocytomas although the clinical course of the two patients was not reported

It is believed that this case report of renal oncocytoma has several hitherto unreported features It is apparently the first such case with a preoperative morphologic diagnosis The tumor cells in smears were somewhat similar to renal tubular cells because of the fine cytoplasmic granules but were much larger In contrast to the frayed cytoplasmic edges of tubular cells the tumor cells were sharply demarcated and were similar in configuration to cells of oncocytomas of salivary gland origin and to oncocytes of thyroid origin [13] The differential diagnosis with renal carcinoma was based on the general appearance of the smears as well as the characteristics of the tumor cells In renal carcinoma of the clear cell type the population of tumor cells in aspiration smears is usually monotonous and abundant and the cells occur in sheets or clusters The cytoplasm of individual cells shows fine vacuoles rather than granules and the nuclei are characterized by the presence of visible enlarged nucleoli The rare renal carcinomas of the granular cell type are characterized by a monotonous population of cells resembling histiocytes with peripheral nuclei and granular cytoplasm [11] Such cells are usually similar in size to normal tubular cells and thus are much smaller than the cells observed in oncocytoma and described here The nature of the tumor was confirmed by ultrastructural examination [11] In our case there were two separate tumor masses one of which extensively invaded the perirenal fat and produced satellite nodules thus documenting the local aggressive behavior and possibly malignant nature of the tumor While evidence for cytologic atypia was only focal and mitotic figures could not be identified the behavior of oncocytic tumors cannot be predicted as shown in the thyroid gland [4]

The nature of oncocytes and of the tumors derived therefrom has been the subject of extensive discussions The presence of masses of large mitochondria within these cells with high levels of ATPase and oxydative enzymes[1] suggests a peculiar transformation of epithelial cells the nature of which is not known In reference to renal oncocytomas their origin from proximal renal tubules was suggested[11] because of their similarity to normal tubular epithelial cells Experimentally oncocytomas may be induced in rat kidneys by nitrosomorpholine [2] The experimental evidence points to an origin of these tumors in the distal renal tubules

In a recent publication on the subject of angiography of renal oxyphilic adenomas Sos et al[12] reported that these tumors are characterized by an avascular or hypovascular appearance and are limited to the renal cortex These authors suggested that following a frozen section confirming the benign nature of the tumor the adenomas can be treated by excisional biopsy only The present case documents the lack of correlation between histologic appearance and tumor behavior and casts serious doubt on the suggested conservative approach to treatment

References

1 Arnold BM Kovacs K Horvath E Murray TM Higgins HP Functioning oxyphil cell adenoma of the parathyroid gland Evidence for secretory activity of oxyphil cells J Clin Endocrinol Metab 38 458 562 1974

2 Bannasch P Krech R Zerban H Morphogenesis and micromorphology of epithelial tumors induced in the rat kidney by nitrosomorpholine III Oncocytic tubules and oncocytomas Z Krebsforsch 92 87 104 1978

3 Blanck C Eneroth CM Jakobsson PA Oncocytoma of the parotid gland Neoplasm or nodular hyperplasia? Cancer 25 919 925 1970

4 Frazel EL Duffy BJ Jr Hurthle-cell cancer of the thyroid Cancer 4 952 956 1951

5 Hamperl H Beitrage zur normalen und pathologischen Histologie menschlicher Speicheldrusen Mikrosk Anat Forsch 27 1 55 1931

6 Hamperl H Benign and malignant oncocytoma Cancer 15 1019 1027 1962

7 Hamperl H Onkocyten und Geschwulste der Speicheldrusen Virch Arch [Pathol Anat] 282 724 736 1931

8 Hamperl H Onkocytes and the so called Hurthle cell tumor Arch Pathol 49 563 567 1950

9 Jaffé RH Adenolymphoma (onkocytoma) of parotid gland *Am J Cancer 16 1415 1423 1932*

10 Klein MJ Valensi QJ Proximal tubular adenomas of kidney with so called oncocytic features Cancer 38 906-914 1976

11 Roth SI Olen E Hausen LS The eosinophilic cells of the parathyroid (oxyphil cells) salivary (oncocytes) and thyroid (Hurthle cells) glands Light and electron microscopic observations Lab Invest 11 933 941 1962

12 Sos TA Gray GF Jr Baltaxe HA The angiographer s appearance of benign renal oxyphilic adenoma *Am J Roentgenol 127 717 722 1976*

13 Zajicek J The aspiration biopsy smear *In* Diagnostic Cytology and Its Histopathologic Bases Third edition Edited by LG Koss *Philadelphia Lippincott 1979 pp 1075-108*

Disseminated Strongyloides stercoralis *Infestation Detected by Sputum Cytology*

Bina Chaudhuri, M D
Stella Nanos, Ph B , C T (ASCP)
Julieta N Soco, C T (ASCP), C T (IAC)
Elizabeth A McGrew, M D , F I A C

This case report is of an immunosuppressed patient in whom a diagnosis of disseminated Strongyloides *infestation was made by cytologic examination of sputum bronchial washing and brushing although a negative bronchial biopsy was reported For those patients receiving steroids or immunosuppressives e important role of cytology in the diagnosis of* rongyloides stercoralis *is emphasized The mor-ologic characteristics of this adult intestinal nema de and the differential diagnosis are also described*

Strongyloides stercoralis is an intestinal nematode commonly found in tropical countries *but rarely in* the *United States* Diagnosis is usually determined by *finding the* characteristic rhabditiform larvae of this nematode in fresh feces However *a characteristic* feature of this roundworm is that it *can cause autoinfection* resulting in widespread dissemination and death in patients treated with corticosteroids and immunosuppressives The case below of disseminated *S stercoralis* in an immunosuppressed *patient reports* a diagnosis made from the cytologic examination of sputum bronchial washing and brushing

Case Report

A 47 year-old Spanish man was admitted to the University of Illinois Hospital with a history of lower gastrointestinal bleeding Four months prior to this admission the patient received a cadaver-donor renal transplant Following the transplant *the patient was treated* twice for acute rejection with high doses of prednisone and azathioprine At the time of admission the patient complained of anorexia generalized body pain and abdominal discomfort He also reported two dark red bowel movements several hours prior to admission

The patient s vital signs were normal Tests showed the lower gastrointestinal tract to be normal Chest X ray revealed a patchy infiltrate involving both lung fields

Specimens of sputum bronchial washing and brushing were sent for cytologic examination to rule out as far as this method permits possible viral infection A bronchial biopsy was also taken for histologic examination although the bronchoscopic examination was normal

Cytologic Findings

Twelve cytologic smears were prepared on glass slides fixed immediately in 95% ethyl alcohol and stained by the Papanicolaou method The examination of slides from sputum bronchial washing and brushing revealed histiocytes ciliated epithelial cells and young adult nematodes (Figures 1 and 2) Each slide contained two to three worms measuring 2 0 mm in length and 40μm to 50μm in breadth (average) The cylindrical muscular esophagus extended through the anterior third of the body and the intestine through the posterior two thirds The caudal end was notched There was no evidence of viral inclusions

The bronchial biopsy originally read as negative was reviewed again after the cytologic findings were

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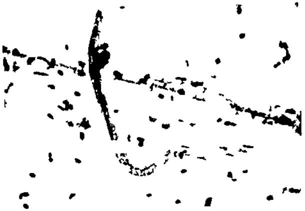

Fig 1
Strongyloides stercoralis detected in sputum cytology (Papanicolaou stain × 400)

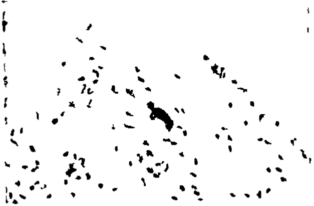

Figure 3
Cut section of *Strongyloides stercoralis* seen in histologic examination of bronchial biopsy (hematoxylin and eosin × 100)

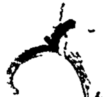

Figure 2
Strongyloides stercoralis found in cytologic examination of material obtained from bronchial brushing (Papanicolaou stain × 400)

reported Fragments of a cross section of worm were seen with no attendant inflammation (Figure 3) The specific diagnosis was made in our parasitology laboratory where numerous rhabditiform and filariform larvae of *S stercoralis* were found on different occasions in the sputum gastric washings and stool

Discussion

Strongyloides stercoralis is an intestinal nematode commonly found in the tropics a few cases have been reported in temperate climates[1] Although rarely found in the United States it is prevalent in areas bordering the southern states and is relatively common in Puerto Rican and other Caribbean immigrants[1] The majority of cases reported in the United States are in immunosuppressed patients e g renal transplant patients[9] those receiving steroids and cytotoxic agents[6,7,10] and those with systemic lupus erythematosus[8] or kwashiorkor[3] Such patients are at risk of developing a potentially lethal autoinfection and may present with generalized infestation[7,8,10] including bronchial involvement[2,4,5] The presence of the infective filariform larvae in bronchial specimens will confirm the diagnosis of an internal autoinfection with this parasite[5]

Most cases of *S stercoralis* are diagnosed by detection of either the filariform larvae or the noninfective rhabditiform larvae in the stool or intestinal content The parasite is easily seen however in specimens of sputum bronchial washings and bronchial brushings when stained by the Papanicolaou method Our *cytologic detection of larvae when bronchial biopsy* results were negative emphasizes the utility of cytology in the diagnosis of this disease especially in immunosuppressed patients it is easier than as well as complementary to the histologic examination Furthermore the intact parasites in the cytologic preparations (Figures 1 and 2) as opposed to the cut sections in the negative bronchial biopsy (Figure 3) simplify the diagnostic procedure

The differential diagnosis should include the larval stages of *Ascaris Necator* and *Ancylostoma* In both hookworms and *S stercoralis* the gullet is open in the rhabditiform stage and closed in the filariform stage In the filariform larval stage however the tail is very pointed in the hookworm but blunt or notched in *S stercoralis*[4,5]

Acknowledgments

The authors would like to thank Ms Carrie James and Dr Susan Davy for their invaluable assistance

References

1 Brown HW Basic Clinical Parasitology Fourth edition New York Appleton Century-Crofts 1975

2 Croll NA Killick Kendrick R Epidemiological significance of *Strongyloides* in sputum Trans R Soc Trop Med Hyg 65 688 1971

3 Grove DI Warren KS Mahmoud AAF Algorithms in the diagnosis and management of exotic diseases III Strongyloidiasis J Infect Dis 131 755 758 1975

4 Johnston WW (editor) Diagnostic cytology seminar Case no 7 Acta Cytol 20 426-428 1976

5 Kenny M Webber CA Diagnosis of strongyloidiasis in Papanicolaou-stained sputum smears Acta Cytol 18 270-273 1974

6 Page FT Reeves DS Accelerated autoinfection with *Strongyloides* species complicating terminal carcinomatosis Southeast Asian J Trop Med Public Health 4 256 259 1973

7 Rassiga AL Lowry JL Forman WB Diffuse pulmonary infection due to *Strongyloides stercoralis* JAMA 230 426-427 1974

8 Rivera E Maldonado N Velez-Garcia E Grillo AJ Malaret G Hyperinfection syndrome with *Strongyloides stercoralis* Ann Intern Med 72 199 204 1970

9 Schumaker JD Band JD Lensmeyer GL Craig WA Thiabendazole treatment of severe strongyloidiasis in a hemodialyzed patient Ann Intern Med 89 644-645 1978

10 Takagi M Ishikawa G Hori E An autopsy case of *Pemphigus vulgaris* complicating a fatal strongyloidiasis Acta Pathol Jpn 23 173 181 1973

Cytologic Features of a Mixed Mesodermal Tumor of the Uterus Demonstrated by Cells Obtained with the Mi-Mark Endometrial Sampler

Peter J Mitchard F I M L S
Gordon R Swingler M B Ch B
Donald G Cave M B

A case is reported of a 68 year old woman with a malignant mixed mesodermal tumor of the uterus Prior to diagnostic curettage and hysterectomy cells were obtained with the Mi Mark Endometrial Sampler Cellular features are described in detail

Mixed mesodermal tumors are reported to account for 0 5% to 5% of all uterine neoplasms[3] and most commonly arise from the bodies of the uteri of postmenopausal women Mixed mesodermal tumors in infants and adolescent women may occur in the cervix and vagina

Histologically these tumors involve the endometrium and the myometrium and demonstrate a mixture of carcinomatous and sarcomatous elements Usually the former is adenocarcinoma and the latter described as either homologous or heterologous In the homologous type round cells resembling leiomyosarcoma or fibrosarcoma are found In the heterologous type mesenchymal elements such as fat bone muscle or cartilage are present Cross striations occur in rhabdomyosarcoma and the differentiation from teratomas is by the limiting of the epithelial elements to those normally occurring in the uterus[1,4,6]

Case Report

A 68 year-old woman (gravida 9 para 8+1) was referred to the gynecologic outpatient clinic with postmenopausal bleeding Menopause had occurred at 53 years of age and postmenopausal bleeding occurred for the first time ten days before referral The patient had mild essential hypertension for which she was being treated with a diuretic but otherwise had had no serious past illness

General examination revealed no significant abnormality On pelvic examination the patient appeared to have a healthy vagina and cervix with a mobile retroverted uterus enlarged to the size of an eight week pregnancy Samples were taken for cytology from the cervix and posterior fornix with an Ayre spatula and from the endometrial cavity with the Mi Mark Endometrial Sampler (Simpson/Basye Inc Wilmington Delaware)[5] The cervical and posterior fornix smears contained anaplastic malignant cells consistent with endometrial carcinoma

Smears were made from the Mi Mark endometrial helix using the collector provided with the sampler kit They were spread rapidly thinly and evenly and wet fixed immediately to avoid drying artifacts The fixative used was technical grade ethanol (98 57 V/V) for a minimum of one hour

Red cell lysing solution was not used because our experience showed that it was unnecessary on thinly spread smears and could adversely affect cellular morphology The smears were stained using a Papanicolaou technique on an automatic stainer

The endometrial smears were retained as one of a

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series assessing the accuracy of the Mi Mark Endometrial Sampler in diagnosing endometrial carcinoma

Insufficient material was obtained with the Mi Mark helix for histologic examination A diagnostic curettage performed under general anesthesia produced many fleshy curettings Histology showed many fragments of carcinoma varying in type from well-differentiated papillary adenocarcinoma to extensive anaplastic carcinoma The appearances were consistent with an endometrial carcinoma

The patient was subsequently treated by cesium insertion to the uterus followed six weeks later by total hysterectomy and bilateral salpingo-oophorectomy There was no evidence of tumor metastasis at laparotomy and she was well eight months later

Results

Gross Pathologic Findings

The uterus and cervix measured 90×55×44 mm Both ovaries and tubes were attached On opening the hysterectomy specimen there was a ragged polypoid mass 20 mm in diameter No other abnormality was found

Histologic Findings

Histology of the tumor showed adenocarcinoma with other areas showing sarcomatous features (Figure 1) In addition foci of chondrosarcoma were seen (Figure 2) In view of the heterologous features it was reported as a mixed mesodermal tumor There was only superficial myometrial involvement but some lymphatic vessels contained malignant cells

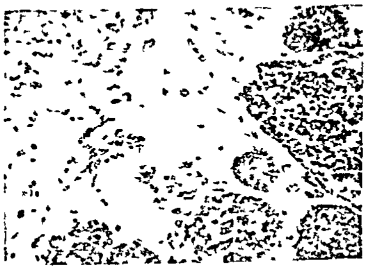
Figure 1
A section of the tumor showing adenocarcinomatous elements (hematoxylin and eosin × 125)

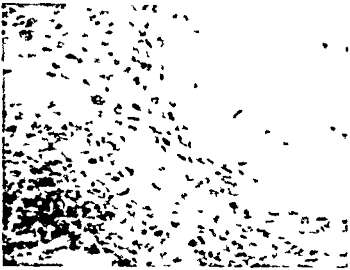
Figure 2
A section of the tumor showing the chondrosarcomatous element (hematoxylin and eosin × 125)

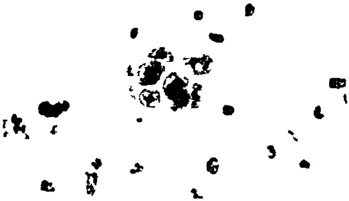
Figure 3
Adenocarcinoma type cells obtained from the Mi Mark sampler (Papanicolaou stain × 500)

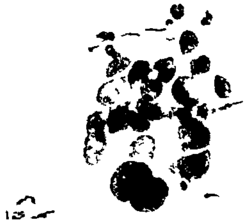
Figure 4
Malignant cells obtained with the Mi Mark sampler showing features suggestive of a mesenchymal origin (Papanicolaou stain × 500)

The remaining endometrium was inflamed and ulcerated The ovaries were atrophic but normal

Cytologic Findings

Figures 3 and 4 show cells which demonstrate the biphasic epithelial and mesenchymal elements found in mixed mesodermal tumors [3,6,8,9]

Figure 3 shows one of the many typical adenocarcinoma type cell groupings Frankly malignant glandular cells such as these had nuclei with coarse clumped malignant chromatin with some condensation of chromatin at the nuclear membrane and prominent nucleoli which often stained red with the Papanicolaou technique They showed much pleomorphism and varying degrees of hyperchromasia together with differing amounts of foamy ill defined basophilic cytoplasm On occasion the cytoplasm was completely absent

Figure 4 shows malignant cells with features suggestive of a mesenchymal origin The nuclei which demonstrate all the criteria of malignancy were oval rather than round with finely granular chromatin one or more nucleoli and a distinct nuclear membrane They showed a variation from clear vesicular to hyperchromatic densely granular forms These cells were generally mononucleate with little or no cytoplasm Where cytoplasm was present it was ill defined delicate basophilic and semitransparent with elongated web like filaments

Present also were poorly preserved tumor cells with round nuclei showing various degrees of hyperchromasia and scanty poorly defined cytoplasm They were suggestive of cells of a stromal origin at different stages of maturation A feature of all the smears examined from this case was the combination of benign atypical and clearly malignant undifferentiated elements this feature was recognized histologically and cytologically as suggestive of a mixed mesodermal tumor

Discussion

Many endometrial samplers have been developed because cervical and posterior fornix smears often fail to detect endometrial malignancy [2] The Mi Mark sampler is designed to scrape the surface of the endometrial cavity to obtain material suitable for cytology In addition fragments of tissue for histologic examination may sometimes be obtained It has the advantage of collecting cells from most of the endometrial cavity whereas endometrial biopsy techniques obtain tissue from only one area In this case of a mixed mesodermal tumor dilatation and curettage under a general anesthetic produced tissue from only the adenocarcinomatous element The Mi Mark sampler however showed two different malignant cell types which suggested the correct diagnosis

Acknowledgments

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References

1 Ackermann LV Rosai J Surgical Pathology Fifth edition St Louis CV Mosby Co 1974 pp 807 808

2 Anderson DG Eaton CJ Galinkin LJ Newton CW Haines JP Miller NF The cytologic diagnosis of endometrial adenocarcinoma Am J Obstet Gynecol 125 376 383 1976

3 Hajdu SI Hajdu EO Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors Philadelphia WB Saunders 1976 pp 183 212

4 Kempson RL Bari W Uterine sarcomas Classification diagnosis and prognosis Hum Pathol 1 331 349 1976

5 M lan AR Markley RL Fi her RS Linthicum CM Witherspoon B Eidschun AG Endometrial cytology using the M lan Markley technique Obstet Gynecol 48 111 116 19 6

6 St rnberg WH Clark WH Smith RC Malignant mixed Mullerian tumor (mixed mesodermal tumor of the uterus) A study of 21 cases Cancer 7 704 724 1954

7 Swingler GR Cave DG M tchard PJ The Mi Mark Endometrial Sampler Diagnostic accuracy in 101 patients with post menopausal bleeding Br J Obstet Gynaecol 86 816-818 1979

8 Tayl CW Mesodermal mixed tumour of the female genital tract J Obstet Gynaecol Br Emp 65 177 181 1958

9 Taylor CW Mullerian mixed tumour Acta Pathol Microbiol Scand [A] [Suppl 3] 80 48 5 1977

Letter to the Editors

46,XY Lung Adenocarcinoma in a 46,XX Female Patient

To the Editors

The recent communication by Genest et al[2] is a striking example of the role which cytogenetics can play in elucidating the basic mechanisms involving cancer They hypothesized that the neoplastic cell line *with the Y chromosome acting as a marker* originated from the patient's father by dispermy and that the neoplastic cell line was repressed until some recent change favored its evolution

An alternative explanation is possible a trophoblast from the pregnancy of a son could have embolized to her lungs[1] and then, when conditions were *favorable* proliferated as a neoplasm This is not however the usual type of trophoblastic tumor

This hypothesis would account for the Y chromosome in the tumor One need not postulate dispermy which did not result in triploidy or a repressed oncogenic factor in the genome emanating from the patient's father who might have had pulmonary cancer *type unknown*

My hypothesis could be checked by performing *cytogenetic marker studies on the tumor the patient* her husband and her sons I suspect that Genest et al would find a concordance of cytogenetic markers *between the tumor and her son*

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References

1 Attwood HD Park WW Embolism to the lungs by trophoblast *J Obstet Gynaecol Br Commonw* 68 611 1961

2 Genest P Lagace R Bouillant A Dumas L Bonenfant J L A 46 XY lung adenocarcinoma in a 46 XX female patient Acta Cytol 23 237 244 1979

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Book Review

Histology A Color Atlas of Cytology Histology and Microscopic Anatomy Second edition revised and enlarged By Frithjof Hammersen Baltimore Urban & Schwarzenberg 1980 $29

Histology A Color Atlas of Cytology Histology and Microscopic Anatomy is as the author states in his preface a visual guide for practical work with the microscope and is intended to teach the student to recognize and differentiate between microscopic structures and to provide guidelines during the practical phase of a histology course With these purposes in mind author Frithjof Hammersen M D Professor and Chairman Department of Anatomy Technical University of Munich has prepared the second edition of the book newly revised and enlarged with 499 illustrations mostly in color

As an atlas the book does not have a long text but this edition does contain in a new introduction a short description of the technical procedures involved in preparing a histologic specimen To supplement this description the author has provided several practical hints on microscope techniques the steps necessary to identify an unknown histologic specimen and spatial conceptions of histologic structures

As the 499 illustrations imply (the first edition had 420) the major purpose of this revision was to enlarge the number of illustrations and to enhance the existing ones from the first edition All of the electron micrographs in the first edition have been replaced by new illustrations several chapters including those on the nervous tissue and the liver include new illustrations And to quote Hammersen again the illustrations from the first edition that originated from invertebrates or non mammals have been replaced in an effort to establish the character of this book as an atlas of *human histology* In all this edition has 79 electron micrographs that do not appear in the first edition This edition also contains 132 new color micrographs plastic semithin sections four new tables and an expanded index

The Editors

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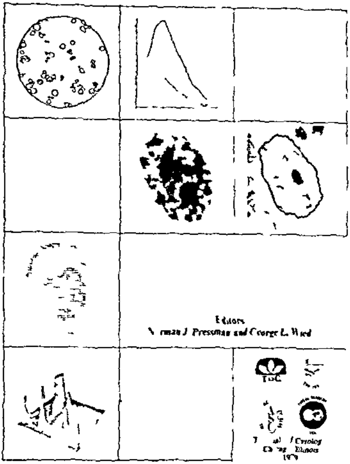

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ACTA CYTOLOGICA
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Editorial

American Cancer Society Report on the Cancer-Related Health Checkup

Steven I Hajdu M D F I A C

The American Society of Cytology is concerned about the American Cancer Society Report on the Cancer Related Health Check up which was released by the American Cancer Society on March 20 1980

One of the major goals of the American Society of Cytology is to detect preneoplastic and neoplastic lesions by using cytologic techniques at the formative stage when such lesions lend themselves to curative treatment at minimal cost This cannot be achieved without well planned and well-coordinated efforts by health professionals professional societies health regulatory agencies and the public The American Society of Cytology considers it unfortunate that the American Cancer Society did not solicit the opinion of concerned professionals or consult with other professional societies prior to the preparation and public presentation of its report

It is disappointing that the American Cancer Society after many years of supporting our efforts recommended that the practice of taking annual "Pap smears" of adult women be discontinued in favor of an examination every three years This recommendation totally ignores the results of screening programs conducted in foreign countries and in the United States that have demonstrated the value of annual cytologic examination in reducing the rate of invasive uterine cervical cancer by the detection and treatment of precancerous lesions The recommendation of the American Cancer Society is potentially

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dangerous because it gives a false sense of security to patients and because an increased number of uterine neoplasms will remain undetected The result will be an apparent rise in invasive neoplasms neoplasms that not only are life threatening but require prolonged treatment

We do not agree with the American Cancer Society that *radiographic and cytologic examinations should* not be recommended for the detection of lung cancer At this time no other techniques are available for the detection of lung cancer at an early and curable stage If these examinations are delayed until symptoms appear as the American Cancer Society recommends then 95% of patients with lung cancer will die

It is apparent that economic considerations played an important role in the guidelines developed by the American Cancer Society but a recommendation to abandon proven cancer prevention measures such *as the annual Pap smear should not be taken lightly* It is the view of the American Society of Cytology that the recommendation of the American Cancer Society, unless modified will have a detrimental effect on the health of the American people

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Fifth International Symposium on Flow Cytometry Clinical and Biological Applications

The Fifth International Symposium on Flow Cytometry Clinical and Biological Applications will be held at the *Orsini-Odescalchi* Castle Bracciano Rome Italy December 2–5 1980 under the sponsorship of the Society for Analytical Cytology The Program Committee consists of F Mauro (Rome) O D Laerum (Bergen) C Nervi (Rome) J Schumann (Munster) and M A Van Dilla (Livermore) For further information write to Symposium on Flow Cytometry c/o I M eR S Via L Bodio 58 00191 Rome Italy

The International Academy of Cytology's Policy Statement on the Frequency of Gynecologic Screening

Recently we have seen publications that attempt to discredit annual cytologic screenings for cervical cancer on the basis of a cost benefit ratio The American Cancer Society very recently has advocated a departure from the established practice of annual screening at least for low risk women

Cytologic screening for uterine cancer has been and is the major stimulus for women to have an annual gynecologic examination including a breast examination This education of our women is in our opinion the principal reason why uterine cancer is a truly preventable disease This is not true for any other major malignant disease

The recommendations made by the American Cancer Society are based on the statistical evaluation of results from screening for cervical cancer of a limited scope They do not define low risk versus high risk women and certainly do not take into account major regional differences as they may exist in various countries Furthermore these recommendations do not take into account changing sexual mores as they have occurred in recent years particularly among very young women who are clearly at risk

It should be evident that the high risk patient will not always come forward and identify herself as such Moreover the clinical history in respect to sexual activities and attitudes is notoriously unreliable It may well be that the cost of an in-depth interview will significantly exceed that of an annual Papanicolaou smear

Another important consideration in evaluating possible extensions of screening intervals is the primary concern with cervical cancer in low risk women It would effectively decrease the frequency of screening in women over 40 years of age in a period of their lives when *endometrial* cancer and its early detection is a major concern

In the United States and in those countries where malpractice suits by patients are widespread a situation of medicolegal interest may develop in which physicians will have to mount a legal defense based merely on the cost-effectiveness considerations of a third party carrier

The cytologic examination provides women with the opportunity for close attention to their general medical needs We do not know of any conscientious physician who would merely obtain a Papanicolaou smear without providing the patient with a general examination including a palpation examination of the breast

The smear besides being a tool of cancer detection is used also for the identification of infections venereal infections (such as *Trichomonas* herpes and *Chlamydia* infections and condylomata) and other alterations as well as for the classification of hormonal patterns The Papanicolaou smear is therefore an integral part of the general diagnostic workup and of good patient care

It must be recognized that the scope of the efficiency of the cytologic examination has never been studied fully The epidemiologists who criticized the procedure are upset because no proper control studies have ever been conducted It is doubtful that such studies could ever take place

Furthermore we recognize that in many societies economically deprived high risk women are more

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dangerous because it gives a false sense of security to patients and because an increased number of uterine neoplasms will remain undetected The result will be an apparent rise in invasive neoplasms neoplasms that not only are life threatening but require prolonged treatment

We do not agree with the American Cancer Society that radiographic and cytologic examinations should not be recommended for the detection of lung cancer At this time no other techniques are available for the detection of lung cancer at an early and curable stage If these examinations are delayed until symptoms appear as the American Cancer Society recommends then 95% of patients with lung cancer will die

It is apparent that economic considerations played an important role in the guidelines developed by the American Cancer Society but a recommendation to abandon proven cancer-prevention measures such as the annual Pap smear should not be taken lightly It is the view of the American Society of Cytology that the recommendation of the American Cancer Society unless modified will have a detrimental effect on the health of the American people

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Fifth International Symposium on *Flow Cytometry* Clinical and Biological Applications

The Fifth International Symposium on *Flow Cytometry* Clinical and Biological Applications will be held at the Orsini-Odescalchi Castle Bracciano Rome Italy December 2-5 1980 under the sponsorship of the Society for Analytical Cytology The Program Committee consists of F Mauro (Rome) O D Laerum (Bergen) C Nervi (Rome) J Schumann (Munster) and M A Van Dilla (Livermore) For further information write to Symposium on Flow Cytometry c/o I M eR S Via L Bodio 58 00191 Rome Italy

The International Academy of Cytology's Policy Statement on the Frequency of Gynecologic Screening

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difficult to reach than their better-endowed sisters

We also recognize that the quality of the sample preparation and interpretation is not always of uniform excellence While we must clearly strive to achieve optimal performance in the clinical and laboratory aspects of cytology it is doubtful that the *departure from the custom of annual examinations* will benefit the patients In fact information available from countries where screening for cervical cancer is performed only at longer than *yearly* intervals clearly indicates that this is not the optimal approach to the control of this disease

Until outstanding quality assurance systems are in place it is our belief that annual screening for cervical cancer represents the optimal approach to the early diagnosis of this disease Finally we believe that the custom of annual examinations must be extended beyond the menopause because evidence is at hand that as the risk for cervical cancer decreases somewhat the risk for endometrial cancer increases sharply Thus it is the latter disease to which our attention must be directed in older women

With these considerations in mind the International Academy of Cytology wishes to reaffirm its recommendation for annual screenings in all women We strongly recommend improvement in the quality of sample preparation and of laboratory quality assurance We also emphatically advise that a great deal of objective modern studies be undertaken prior to the introduction of any major changes in the medical care of our patients

The International Academy of Cytology

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The Paradox of Sarcomas

Steven I Hajdu M D F I A C

The better understanding of sarcomas was held back for centuries by among other things misuse and misunderstanding of various names and definitions Galen who lived and worked in the second century defined sarcomas in such a way that any form of inflammatory or infectious swelling as well as all sorts of benign tumors was called a sarcoma

As late as the 1800s some physicians believed that sarcomas did not possess malignant tendencies Even today in fact a breast tumor (cystosarcoma phyllodes) that is a benign neoplasm in 95% of the cases is referred to as a sarcoma It is of importance that despite 2 000 years of history of European medicine the first comparative description of sarcomas called *encephaloid tumors* and carcinomas called *scirrhus tumors* was done by Samuel Gross in 1866 (Table I)[3] He was a native of Laston Pennsylvania and professor of surgery at Jefferson University in Philadelphia His system of classification was modified by James Ewing[2] pathologist at Memorial Hospital for Cancer and Allied Diseases in New York in 1919 Ewing s classification system served as the basis for our present classification of sarcomas[4,7,11]

Sarcomas because of their bulky and disfiguring nature were illustrated in medical publications much earlier than carcinomas (Figure 1)

Practically all malignant tumors occurring in patients under age 16 are nonepithelial in origin[7] Sarcomas with a few exceptions are found most commonly in males (Table II) soft tissue as well as bone sarcomas have a unique site preference for the extremities[3,7,11]

The cell of origin of fibroblasts is far from settled

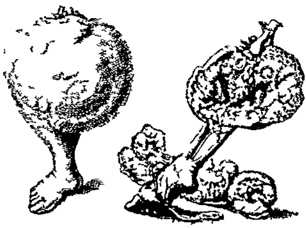

Figure 1
Engraving of a sarcoma published in the *Philosophical Transactions* vol IX p 271 London 1747

From the Department of Pathology Memorial Sloan Kettering Cancer Center New York New York U S A

Dr Hajdu is Attending Pathologist and Chief of the Cytology Service Memorial Sloan Kettering Cancer Center and Associate Professor of Pathology Cornell University Medical College New York New York

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Table I *Differential Diagnosis of Sarcomas and Carcinomas*

Encephaloid	Scirrhus
1 The tumor is soft and elastic not uniformly but more so at some points than at others	1 Ultimately hard and inelastic feeling like a marble beneath the skin
2 It grows rapidly and soon acquires a large bulk perhaps ultimately attaining the volume of an adult s head	2 Growth is slow and bulk comparatively small the tumor rarely even in the worst cases exceeding the volume of a double fist
3 The pain is slight and erratic until ulceration begins when it becomes more severe and fixed	3 The pain begins early is distinctly localized and is of a sharp darting burning or lancinating character
4 There is always a marked enlargement of the subcutaneous veins	4 In scirrhus these vessels retain their natural size or are only slightly enlarged
5 The ulcer is foul and fungous with thin undermined and livid edges and is subject to frequent and copious hemorrhage	5 The ulcer is incrusted with spoiled lymph and has steep abrupt edges looking as if it has been scooped out of the part bleeding little and seldom
6 There is generally early lymphatic involvement	6 Usually not until late or just before ulceration is about to occur
7 Occurs at all periods of life	7 Seldom before the age of forty five
8 Is most frequent in the eye testicle mamma lymphatic ganglions bones skin and cellular tissue	8 Never occurs in the eye and testicle and rarely in the bones skin and lymphatic ganglions
9 The disease usually terminates fatally in from nine to twelve months	9 Seldom sooner than eighteen months or two years

From Gross A System of Surgery Philadelphia HC Lea 1866

Table II *Sex Prevalence of Sarcomas*

Male	Female
Malignant fibrous histiocytoma	Lymphangiosarcoma
Fibrosarcoma	Malignant schwannoma
Tendosynovial sarcoma	Malignant glandular cell tumor
Liposarcoma	Alveolar soft part sarcoma
Rhabdomyosarcoma	
Kaposi s sarcoma	
Osteogenic sarcoma	
Chondrosarcoma	

Modified from Hajdu Pathology of Soft Tissue Tumors Philadelphia Lea & Febiger 1979

but most investigators believe that fibroblasts derive from primitive pluripotential mesenchymal cells [4,7,20] Such cells in the course of differentiation become specialized and may assume the cytologic characteristics of chondroblasts osteoblasts myoblasts lipoblasts angioblasts and other primitive forms (Figure 2) Despite Carrel s 1926 warning that connective *tissue cells like bacteria must be defined not only by their morphology but also by their physiological properties* mesenchymal cells and mesenchymal tumors have rarely been studied from any point of view other than pure morphology

Fibroblasts are characterized by the production of reticulin and collagen The transition from reticulin to collagen is gradual and continuous Collagen is a polypeptide and varies according to the number and kind of the constituent amino acids the manner of union of radicals and the polymerization of molecules The association of collagen fibers into bundles is related to the biosynthetic activity of the fibroblasts which determines their architectural and mechanical properties [4-7] For example tendons and collagenous fibrous tumors such as *fibrosarcomas* contain type I collagen fibers which stain blue with trichrome stain and appear as both intracellular and extracellular granular material The thin type III collagen fibers which are positive with reticulin stain are found in vessels muscles and parenchymal tissues The polymeric type II collagen is found in cartilage [4]

The pathway of collagen production is complex and is believed to be carried out in fibroblasts simultaneously with the synthesis of polysaccharides The quantity of collagen polysaccharides and glycosaminoglycans varies from tumor to tumor and from anatomic site to anatomic site Heavily collagenous sarcomas are relatively low-grade neoplasms histologi

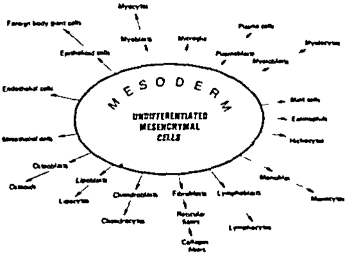


Figure 2
Histogenesis and differentiation of various mesenchymal cells

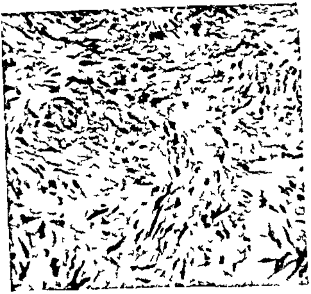


Figure 3
Desmoid tumor composed of uniform collagenous fibroblasts (hematoxylin and eosin × 140)

cally e g desmoid tumors (Figure 3) but they may be dangerous because of their anatomic location near vital structures

Noncollagenous soft tissue and bone neoplasms so-called *fibrous histiocytomas* are known for their extreme variation in gross appearance microscopic pattern and clinical behavior Perhaps one of the most striking peculiarities of fibrous histiocytomas is the large number of binucleated Reed Sternberg-cell like and multinucleated epulis or Touton giant cells There is substantial evidence from autoradiographic cytogenetic cell kinetic and ultrastructural studies that multinucleated cells are the result of fusion of mononuclear cells and are not produced by amitotic division Amitotic division would require the presence of pairs of centrioles close to each of the nuclei within multinucleated cells at all times

Mitotic activity in fused cells is unlikely because the centrioles are piled up in a giant centrosphere in the nucleus-free area of the cytoplasm Histiocytic giant cell formation is an important and efficient disposal system for unwanted cells ones that may be potentially dangerous or harmful to the host[4,7]

It is known to morphologists that mesenchymal tumors that contain large numbers of epulis Langhans s and/or Touton giant cells have a benign course but that when such cells are absent or few in number the prognosis of fibrous histiocytic tumors should be regarded as guarded (Figure 4)

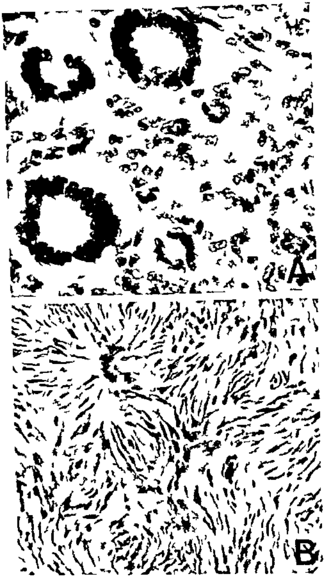


Figure 4
(A) Benign pleomorphic fibrous histiocytoma (so-called xanthoma) composed of mononucleated stromal cells and multinucleated Touton giant cells (hematoxylin and eosin × 350) (B) Malignant fibroblastic fibrous histiocytoma (so-called dermatofibrosarcoma protuberans) composed of uniform noncollagenous fibroblasts The storiform pattern is a characteristic feature of fibrous histiocytomas (hematoxylin and eosin × 140)

The so-called histiocytes in histiocytic fibrous tumors are functionally active mesenchymal cells and may derive as undifferentiated forms from primitive cells e g fibroblasts pericytes and Schwann cells

Mucopolysaccharides constitute an important part of the ground substance in sarcomas Most of the biochemically identified mucopolysaccharides are in cluded in the term *heteroglycan* which includes the glucosaminoglycans hyaluronic acid chondroitin sulfate and heparin

Hyaluronidase sensitive mucopolysaccharides have been demonstrated in myxoid soft tissue tumors but myxoid chondrosarcomas have been known to contain mostly hyaluronidase resistant mucosubstances[2] Periodic acid Schiff (PAS) stain has been found useful to separate diastase-sensitive glycogen which is present in soft tissue tumors from diastase-stable material commonly seen in certain bone tumors and epithelial neoplasms[4,7,11,2]

Most sarcomas contain S antigen demonstrable by indirect immunofluorescence with human sera Considerable effort is presently being made by a number of investigators to detect specific sarcoma associated antigens[16] Sethi and Hirshaut have reported not only the presence of specific antigens but also the elevation of the level of circulating antibodies to S antigens in patients after surgical removal of soft tissue sarcomas[17]

The normal development of muscle progresses from small round cells (myoblasts) through a spindle cell form (myocytes) to multinucleated muscle fiber Longitudinal and transverse striations appear at the most advanced stage of differentiation The nuclei of benign as well as neoplastic muscle cells are located in the periphery of the muscle fiber adjacent to the sarcolemma Multinucleated muscle fibers are formed by the fusion of adjacent cells Fusion is initiated by recognition of homotypic cells and is mediated by specific molecular markers on the cell surface The characteristic dark and light banding of each myofibril which gives the typical striated pattern of the muscle fiber is due to the highly ordered and repetitious arrangement of myofilaments and is rarely seen in myosarcomas (Figure 5)

Although electron microscopic observation offers little aid in predicting whether a muscle tumor is benign or malignant it helps to establish the myogenic origin of many neoplasms that cannot be classified by any other technique

It has been shown[4,17] that various sarcomas share a common antigen and that they are unable to react with specific antibodies when tissue sections are examined in an immunofluorescence system It is apparent that neoplastic cells of origin other than muscle may show reactivity with muscle antibody since actinlike contractile microfilaments may appear in fibroblasts and other mesenchymal cells when the cells are stimulated by cell to-cell contact It is of interest that a heavy buildup of intracytoplasmic myofilaments is seen in mesenchymal cells following the administration of vincristine and some other chemotherapeutic agents[4]

It has been estimated that about 10% of all sarcomas of soft tissues are *rhabdomyosarcomas*[4,7] The relative rarity of rhabdomyosarcomas is partly explained by the fact that striated muscle cells are dif

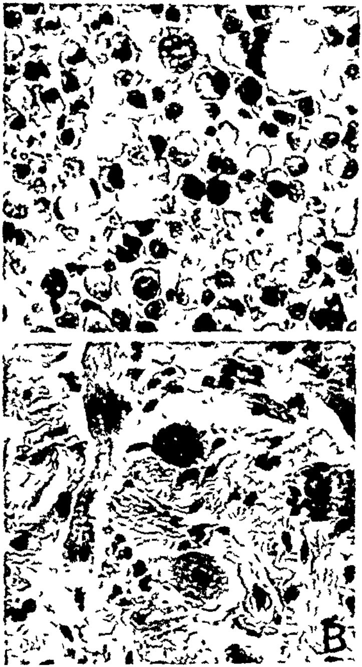


Figure 5
(A) Embryonal rhabdomyosarcoma composed of round and oval malignant rhabdomyoblasts Fine intracytoplasmic filaments are visible in many rhabdomyoblasts (hematoxylin and eosin × 350)
(B) Pleomorphic rhabdomyosarcoma showing longitudinal and cross-striated cytoplasmic filaments (hematoxylin and eosin × 350)

ferentiated out-of-cycle cells and do not undergo mitoses in postnatal life It is astonishing considering the weight and extent of skeletal muscle in the body that it has fewer tumors than any other tissues Apparently most of the rhabdomyosarcomas arise from undifferentiated mesoderm rather than from myotome-derived skeletal muscle

Embryonal rhabdomyosarcoma is the most common soft tissue sarcoma in children and an estimated one third of embryonal rhabdomyosarcomas are primary neoplasms of the head and neck region [4,7,8] It is indeed a paradox that rhabdomyosarcoma is prone to occur in children where striated muscle is not usually found e g urinary bladder vagina and prostate The origin of striated muscle tumors in organs in which no striated muscle is normally found has not been explained to everybody s satisfaction (Figure 5A)

On the other hand pleomorphic rhabodmyosarcoma is predominantly a disease of adults and predominantly affects males during the fifth decade It is usually a painless neoplasm of an extremity and is considered one of the most malignant forms of sarcoma Microscopically pleomorphic rhabdomyosarcomas are composed of pleomorphic rhabdomyoblasts which stain acidophilic with hematoxylin and eosin and red with PAS and trichrome stains (Figure 5B)

Although adipose tissue constitutes in normal conditions about 15% to 25% of the human body (and in obese individuals fat may contribute more than 50% of the body weight) it received little attention prior to the 1960s when a number of publications appeared on the physiology and biochemistry of adipose tissue The adipose tissue is a specialized tissue an organ on its own and is composed of four distinct cells glycogen containing fibroblastlike *prolipoblasts* round granular cytoplasmic *lipoblasts* multivacuolated *preadipocytes* and mature fat cells (*adipocytes*) with globoid fat vacuoles [4,8] The differentiation of fat cells can best be appreciated by electron microscopic examination of fat cells at various stages of maturation from immature to mature forms [4]

The cell of origin of the fat cell is unknown Ewing in a lecture in 1935 said that steatoblasts and lipoblasts are derived from the adventitial layers of the blood vessels Capillary vessels are indeed close to the surface membrane of fat cells and a branching capillary network resembling chicken wire can be clearly seen in the majority of liposarcomas (Figure 6)

Lipomas benign adipose tissue tumors and liposarcomas malignant tumors of adipose tissue are the bulkiest and the most common soft tissue neoplasms (Figure 7)

The first reference to adipose tissue tumors is in the *Papyrus Ebers* which was written in 1552 B C The largest adipose tissue tumor was reported and illustrated in the *Cleveland Medical Gazette* in 1859 Ac

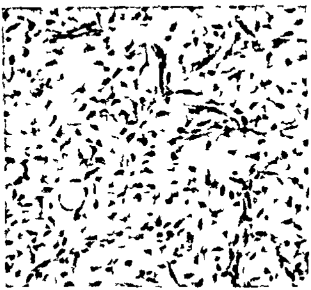


Figure 6
Myxoid liposarcoma composed of finely vacuolated preadipocytes Note the branching capillary network (hematoxylin and eosin × 140)

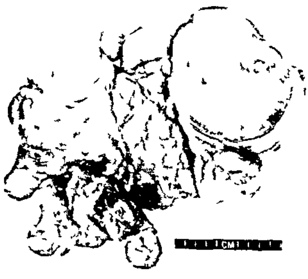
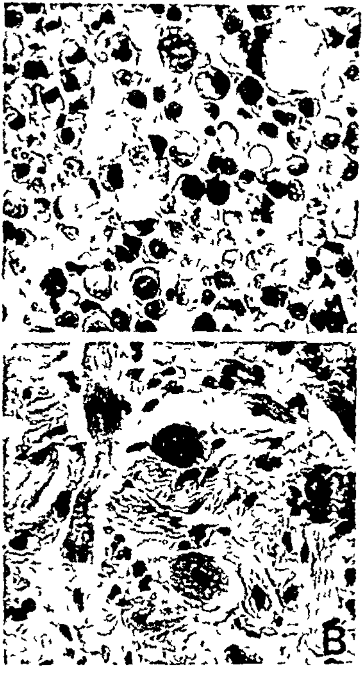


Figure 7
This huge multilobular liposarcoma was excised from the retroperitoneum

cluded in the term *heteroglycan*, which includes the glucosaminoglycans, hyaluronic acid, chondroitin sulfate and heparin.

Hyaluronidase sensitive mucopolysaccharides have been demonstrated in myxoid soft tissue tumors, but myxoid chondrosarcomas have been known to contain mostly hyaluronidase resistant mucosubstances.[22] Periodic acid Schiff (PAS) stain has been found useful to separate diastase-sensitive glycogen, which is present in soft tissue tumors, from diastase-stable material, commonly seen in certain bone tumors and epithelial neoplasms.[4,7,11,22]



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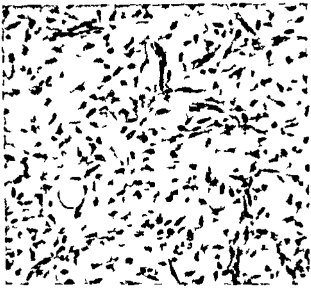

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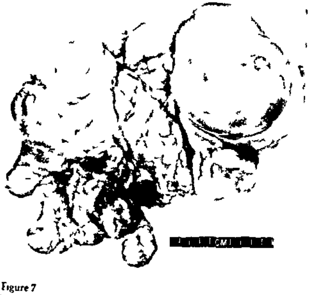


Figure 7
This huge multilobular liposarcoma was excised from the retroperitoneum

cording to historians[4] Delameter travelled on horseback from Cleveland to the area of Dayton Ohio where he operated on the patient and removed a 268 pound tumor

Histologically five different types of liposarcomas can be distinguished *well differentiated myxoid lipoblastic fibroblastic* and *pleomorphic* Fortunately most of these tumors despite their bulky nature are relatively low-grade neoplasms It is perhaps important to point out that the grade of malignancy of liposarcomas just like that of most other sarcomas is assessed by considering the cytomorphology of the tumor cells[7,23]

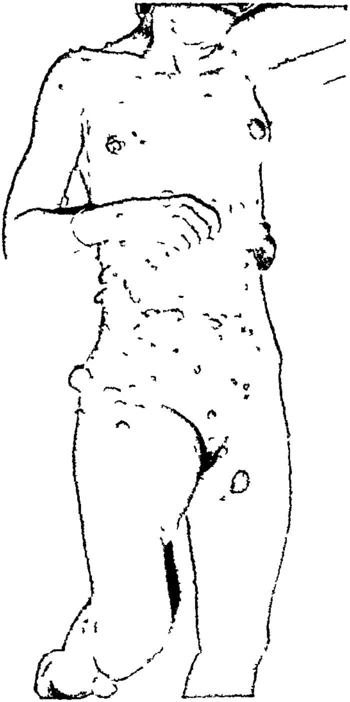


Figure 8
This 39 year-old woman had multiple neurofibromas and cafe-au lait spots from childhood

In 1872 Moritz Kaposi a Hungarian physician working in Vienna gave the first account of an *angiosarcoma* which was later named after him[12] (Kaposi s original name was Moritz Kohn but being very much in love he changed his name to a non Jewish one when his would be father in law Professor von Hebra the famous German dermatologist told him that his daughter could not marry a Jew)

Kaposi s sarcoma has a worldwide distribution but it has a racial propensity for the male Jewish and Italian populations of Eastern Europe and Russia An additional interesting and puzzling feature of Kaposi s sarcoma is its frequent association with malignant *lymphoreticular neoplasms particularly with* Hodgkin s disease[4] The mortality from the second primary cancer is a greater threat to patients with Kaposi s sarcoma than is that from Kaposi s sarcoma

Neurofibromatosis or von Recklinghausen s disease[4,19,21] is a progressive hereditary disease and is directly related to the proliferation of Schwann cells It can affect any age sex race or color Cutaneous neurofibromas are most apparent on the trunk and head and neck areas and are frequently associated *with frecklelike discolorations or cafe au lait spots* (Figure 8) Most of these tumors remain unchanged for several years but some of the tumor nodules may burst into sudden growth reach enormous size and transform into a highly malignant tumor collagenous malignant *schwannoma* or so-called *neurofibrosarcoma* It is of interest that noncollagenous malignant schwannomas are rarely found in association with von Recklinghausen s disease and in most instances are self limited and low-grade neoplasms[4,19]

Clinically sarcomas especially *bone sarcomas* may present in a very insidious way and their detection may require careful x ray examination[10,11] They *may appear as a small nodule* requiring surgical exploration by a skilled surgeon because they may be hidden in muscle compartments (Figure 9A) others may be subcutaneous fungating (Figure 9B) and multifocal (Table III)

Table III The Most Common Multifocal Sarcomas

Epithelioid sarcoma
Angiosarcoma
Neurofibrosarcoma
Kaposi s sarcoma
Granulocytic sarcoma

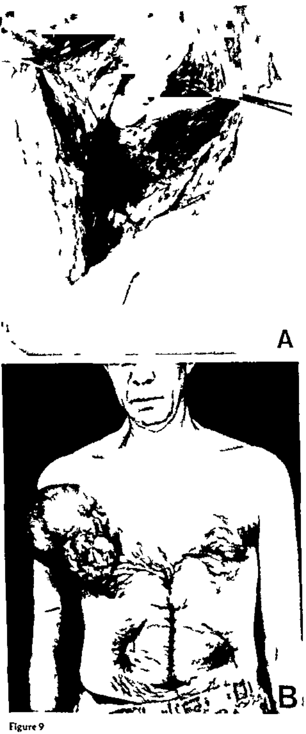


Figure 9
(A) This small but high-grade fibrosarcoma was found deep in the right thigh (B) A bulky and fungating dermatofibrosarcoma protuberans low-grade malignant fibrous histiocytoma of the right upper chest wall

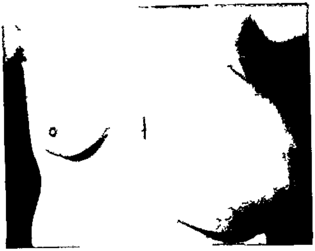


Figure 10
Benign cystosarcoma phyllodes of the left breast

Without microscopic examination the gross appearance of connective tissue neoplasms can be misleading because some of the bulky tumors are true sarcomas many others however e g cystosarcoma phyllodes (Figure 10) and neurofibroma are benign neoplasms despite their large size

The *microscopic appearance* of sarcomas is in direct contrast to their ugly gross features Consider e g the beautiful storiform pattern of fibroblastic fibrous histiocytoma (Figure 4B) the parallel arrangement of peripheral nerve tumors of a neurofibrosarcoma (Figure 11A) or the monotonous lacy growth of alveolar rhabdomyosarcoma and myxoid chondroid neoplasms (Figure 11B)

The *cytologic appearance* of individual tumor cells can also be misleading [4,7,8,10] Neoplastic cells of Ewing s sarcoma may resemble those of oat cell carcinoma (Figure 12A) Exfoliated cells of tendosynovial sarcoma may have features of glandular cells (Figure 12B)

Exfoliated cells of osteogenic sarcoma in sputum can be so squamoid that even the most experienced cytologists can be fooled by their appearance [7,10] Features that are trademarks of sarcoma cells are their bizarre shape and size Consider for example clusters of tumor cells of pleomorphic fibrous histiocytoma (Figure 13A) or the cigar-shaped exfoliated cells of leiomyosarcoma (Figure 13B) with their bluish ill-defined cytoplasm No cells are bigger or more colorful than the giant epulis cells (Figure 13C) and the bizarre cells of pleomorphic rhabdomyosarcoma (Figure 13D)

In 1903 Woglom said Every one whose experi

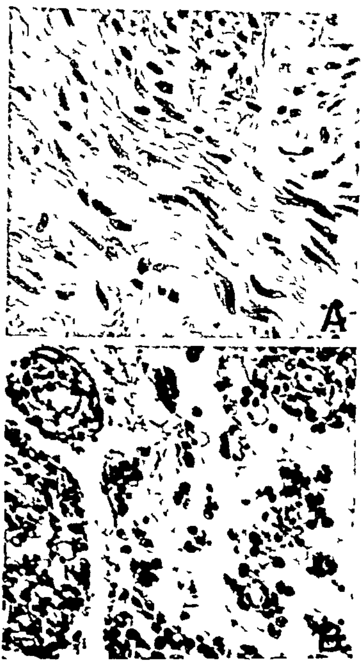


Figure 11
(A) Well-differentiated neurofibrosarcoma showing parallel rows of neoplastic cells (hematoxylin and eosin × 140) (B) Chordoid sarcoma composed of *uniform stellate cells arranged in lacy clusters* (hematoxylin and eosin × 140)

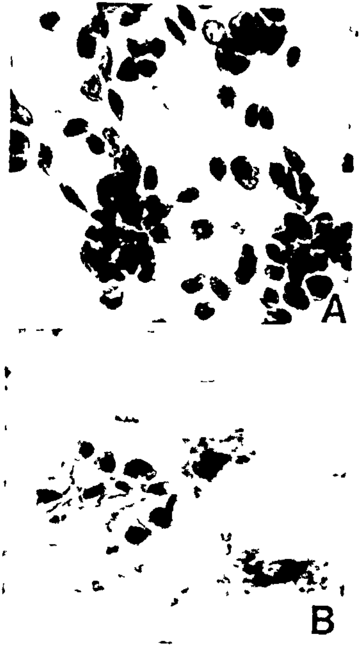


Figure 12
(A) Clusters of small pyknotic cells of Ewing's sarcoma in aspiration smear (hematoxylin and eosin × 450) (B) Cluster of epithelioid *tumor cells of metastatic tendosynovial sarcoma in sputum* (Papanicolaou stain × 570)

ence has been limited to the investigation of human material must have felt dissatisfaction at one time or another with the inconclusiveness of his observations [23] And he added with foresight the value of working with a tumor which can be propagated in animals through many generations is apparent we can alter at will the conditions under which it is growing and we can submit it to examination at any period of its growth

In 1911 Payton Rous in the remarkable experiment for which he received a Nobel prize in 1966 proved that a tumor a *sarcoma* could be transferred in chickens by the inoculation of cell free infiltrate [19] It is of interest also that the first human tumor grown in tissue culture by Losee and Ebeling in 1914 [11] was also a sarcoma an osteogenic one

We reported several years ago that mobilization of tumor cells grown *in vitro* alters the cell morphology and for example that spindle cells become round and assume cytologic features of exfoliated cells [3]

It belongs to the paradox of sarcomas that human sarcomas grafted into athymic nude mice at different

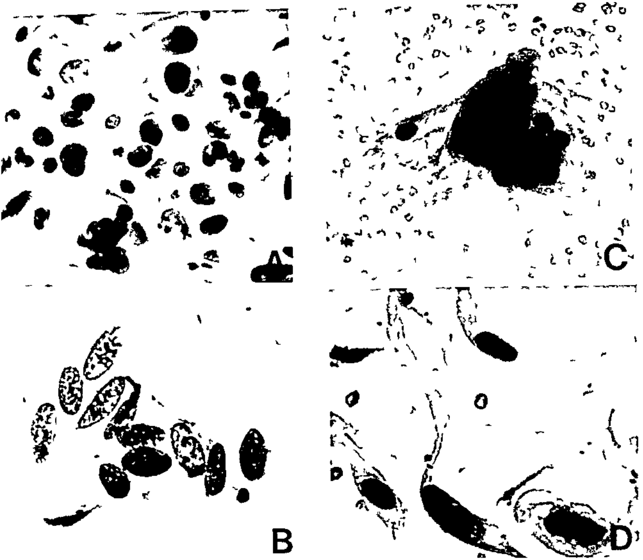
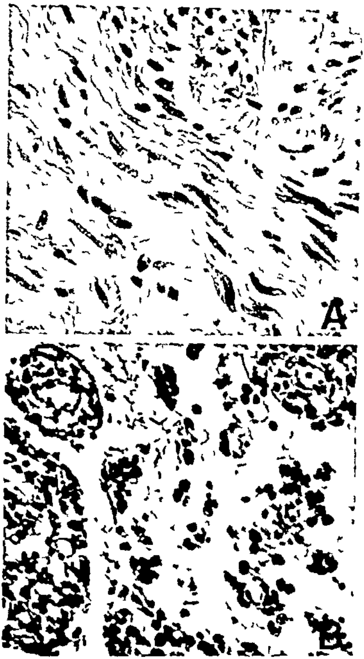


Figure 13
(A) Neoplastic cells of malignant pleomorphic fibrous histiocytoma in aspiration smear (hematoxylin and eosin × 450) (B) Imprint smear prepared from leiomyosarcoma of the uterus showing uniform cigar-shaped malignant leiomyoblasts Note the ill-defined and scanty cytoplasm (Papanicolaou stain × 570) (C) Epulis type multinucleated giant cell showing pseudopodlike cytoplasmic extension in aspiration smear of giant cell tumor of bone (hematoxylin and eosin × 450) (D) Strap cells of pleomorphic rhabdomyosarcoma with long cytoplasmic processes in aspiration smear (hematoxylin and eosin × 450)

sites produce tumors that show a spectrum of morphologic variations [4]

As more and more cancer patients receive irradiation therapy and survive the danger of *radiation induced sarcoma* becomes increasingly possible [4,11] Tumors that may develop following therapeutic irradiation or incidential radiation include various epithelial and lymphoreticular neoplasms It is less well known that radiation often affects soft tissues and bones (Table IV) The paradox of irradiation sarcomas is the long period of precancerous changes in the tissues which may last for years or decades yet once the sarcoma appears there is a fast downhill course and most of the patients die in less than two years after the diagnosis of sarcoma is made

In the *treatment of sarcomas* amputation was a leading therapeutic measure for centuries Scapulohumeral amputation and disarticulation was introduced by accident in Holland in the 17th century[1] the arm of a man was pulled by the wheel of a windmill this gave surgeons the idea of disarticulation [4]



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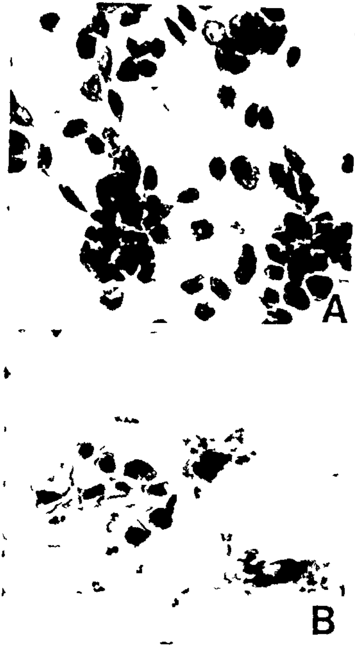

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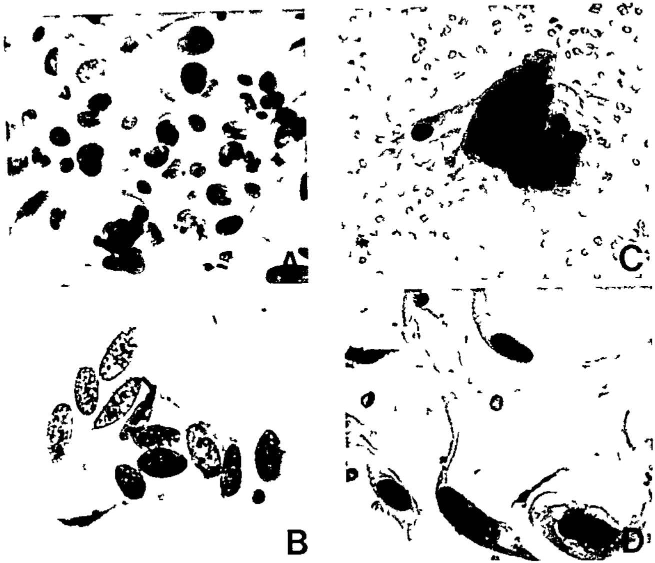

Figure 13
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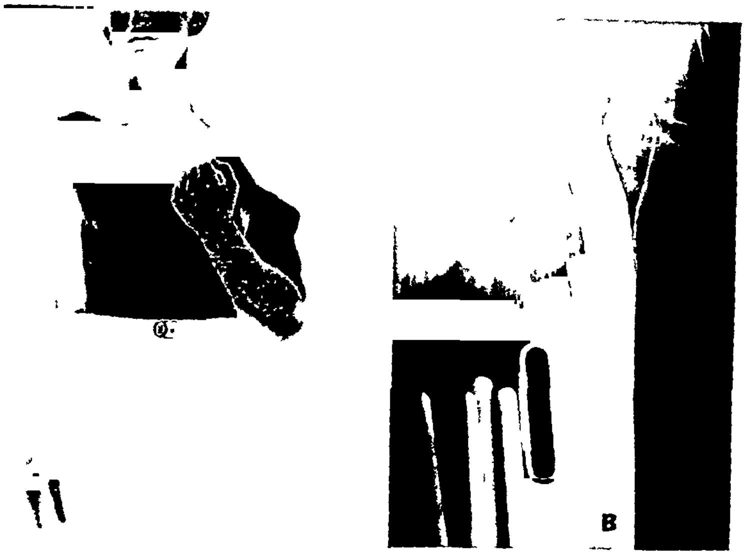

igure 14
A) Fibrosarcoma of the shoulder resected in such a way that the rm was saved (B) Left lower extremity saved by replacing the mur with a metal prosthesis following resection of sarcoma

The most heroic attempts to cure sarcomas with mutilating surgery including hemicorporectomy were practiced until the 1960s The present treatment of sarcomas is limited limb-saving surgery (igure 14A) combined with irradiation and chemotherapy Limb-saving operation with replacement of vital tructures such as vessels and bones with plastic and metal prostheses (Figure 14B) makes amputation unnecessary and ensures satisfactory function Today amputation is used only when vital strucures such as major vessels and nerves are complete y compromised by the sarcoma[4,11,15,18]

The outlook for patients with sarcoma has hanged drastically for the better during the last ten ears The mortality rate significantly decreased durng the last decade and it can be claimed that more rogress has been made during the last ten years in

Table IV *Radiation Induced Soft Tissue and Bone Sarcomas*

	No of patients	Median elapsed time (years) between irradiation and appearance of sarcoma	No of patients who died of disease
Soft tissues	38	13	32
Bones	29	11	22

Personal experience

Table V *Comparison of Five-Year Case Fatality of Certain Sarcomas*

	Average mortality (%)	
	in 1968	in 1978
Osteogenic sarcoma	90	55
Leukemias	90	40
Ewing s sarcoma	85	25
Malignant lymphomas	75	40
Embryonal rhabdomyosarcoma	75	30
Wilms s tumor	75	20

the treatment of sarcoma than any other neoplasm (Table V)[4,7,11,18]

Undoubtedly the multidisciplinary management of sarcomas by surgeons radiation therapists and medical oncologists is a major factor in the changing prognosis of sarcomas but let us not forget the thousands of workers including cell biologists cytologists and pathologists without whom the better understanding of these neoplasms and the advance in therapy could not have been made

References

1 Cheselden W The Anatomy of the Human Body S th edition Londres Bowyer 1741

2 Ewing J Neoplastic Diseases Philadelphia WB Saunders 1919

3 Gross SD A System of Surgery Pathological Diagnostic Therapeutic and Operative Fourth edition Philadelphia HC Lea 1866

4 Hajdu SI Pathology of Soft Tissue Tumors Philadelphia Lea & Febiger 1979

5 Hajdu SI Bean MA Hajdu EO Ricci A Papanicolaou smears of cultured human tumor cells Acta Cytol 18 327 332 1974

6 Hajdu SI Erlandson R Fortner JG Classification and histologic grading of liposarcomas Cancer (in press)

7 Hajdu SI Hajdu EO Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors Philadelphia WB Saunders 1976

8 Hajdu SI Koss LG Diagnosis of metastatic myosarcomas Acta Cytol 13 545 551 1969

9 Hajdu SI Lemos LB Kozakewich H Helson L Beattie EJ Growth pattern and differentiation of human soft tissue sarcomas in nude mice Cancer (in press)

10 Hajdu SI Melamed MR Needle biopsy of primary malignant bone tumors Surg Gynecol Obstet 133 829-832 1971

11 Huvos AG Bone Tumors Diagnosis Treatment and Prognosis Philadelphia WB Saunders 1979

12 Kaposi M Idiopathisches multiples pigment-sarkom der Haut Arch Dermatol Syphil 4 265 1872

13 Losee JR Ebeling AH The cultivation of human sarcomatous tissues *in vitro* J Exp Med 20 140-148 1914

14 Pertschuk LP Immunofluorescence of soft tissue tumors with anti-skeletal muscle antibodies Am J Clin Pathol 63 332 34- 1975

15 Rosen G Management of malignant bone tumors in children and adolescents Ped atr Clin Am 23 183 13 1976

16 Rous FP A transmissible avian neoplasm (sarcoma of the common fowl) J Exp Med 12 696 705 1910

17 Sethi J Hirshaut Y Complement-fixing antigen of human sarcomas J Natl Cancer Inst 57 489-493 1976

18 Shiu MH Magill GB Hopfan S Recent trends in treatment of soft tissue sarcomas *In* Pathology of Soft Tissue Tumors Edited by SI Hajdu Philadelphia Lea & Febiger 1979

19 Sordillo PP Helson L Hajdu SI Magill GB G[illegible] RB Beattie EJ Malignant schwannoma Clinical characteristics [illegible] val and response to therapy Cancer (in press)

20 Stout AP Tumors of Soft Tissues Washington DC Armed Forces Institute of Pathology 1953

21 von Recklinghausen FE Über die multiple Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen Berlin Hirschwald 1882

22 Winslow D E[illegible] FM [illegible] and mucopolysaccharides in [illegible] Am J Pathol [illegible] 19[illegible]

23 [illegible] The [illegible] Cancer [illegible] Columbia University Press [illegible]

Cytology of Common Primary Midline Brain Tumors

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Jick Yee Wong M S C T (ASCP),
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Common primary midline brain tumors can be adequately diagnosed by cytologic methods if their results are interpreted in the light of the clinical setting and the neuroradiologic findings The type of cytologic material appropriate for diagnosis will vary with the type of lesion Cerebrospinal fluid (CSF) cytology is considered useful for the diagnosis of malignant teratomatous lesions which frequently include an abundant germinomatous (seminomatous) element since these tumors readily exfoliate into the CSF In other common lesions (craniopharyngiomas and pituitary adenomas) direct aspiration methods are thought to be more applicable

Aspiration cytology was unable to provide a definitive diagnosis in two midline cystic lesions both apparently benign and it is suggested that the method may have certain drawbacks in differentiating such lesions

Nerve tissue neoplasms which not infrequently arise in the midline structures were not included in this series

Primary neoplastic lesions occurring in the midline region of the brain along the pineal pituitary axis include gliomas pineal tumors usually of the germ cell teratomatous type with a higher incidence of those with a germinomatous (seminomatous) element craniopharyngiomas and their cystic variants pituitary adenomas commonly of the chromophone variety ependymomas and cysts either dermoid ependymal or arachnoidal[10 15] These lesions with some exceptions (pituitary adenomas) tend to affect the young and in many there is a male preponderance (midline teratomas craniopharyngiomas)

These lesions present with common symptoms because they obstruct the ventricular system producing obstructive hydrocephalus and compressing vital midline structures such as the quadrigeminal plate third ventricle hypothalamus infundibulum pituitary gland and optic chiasm Their initial identification is based on age prevalence clinical presentation and the results of improved neuroradiologic studies which provide information on their location pattern of growth type of expansion and consistency (solid vs cystic presence of calcifications etc) The absence of a primary malignancy elsewhere in the body becomes at times useful information for their characterization Their final diagnosis however rests on histologic proof by appropriate biopsy The purpose of this report is to report the role of exfoliative cytology of CSF and needle aspiration cytology in the diagnosis of such lesions

Materials and Methods

The present report is based on cytologic experience obtained during the past nine years with 11 cases 10 of which were adequate diagnosed These ten consisted of primary teratomatous and epithelial lesions including three germinomas (one admixed with other teratomatous elements) five craniopharyngiomas (including a benign cystic variant) and two pituitary chromophobe adenomas

The location and growth pattern of these lesions were delineated by neuroradiologic studies which in many cases included computerized tomographic (CT) scan with contrast

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Table I presents the clinical and laboratory diagnostic data on these cases with reference to the type of clinical presentation the neuroradiologic findings other than obstructive hydrocephalus with the tentative diagnosis (when CT scan was used this is indicated) the results of previous tissue biopsy if such existed the type of cytologic diagnostic method used and its results and the final histologic diagnosis The last was defined by the results of a review of previous histology a more recent surgical biopsy or postmortem examination In two cases of craniopharyngioma the tumor was inaccessible to biopsy and the final diagnosis rested on the cytologic findings

In the three cases of germinoma CSF specimens obtained by lumbar puncture were used In the craniopharyngiomas and the pituitary adenomas the cytologic material was obtained by direct needle aspiration of the lesions This sample was submitted to the cytology laboratory as a liquid specimen in aspirations of cystic lesions or as a rinsate in normal saline in aspirations of solid lesions The specimens were processed according to their consistency utilizing spreads cytocentrifuge preparations and membrane filter preparations (5 0μm Nuclepore or Millipore filters Nuclepore Corporation Pleasanton California and Millipore Corporation Bedford Massachusetts respectively)

After fixation in 95% ethyl alcohol these preparations were stained by the Papanicolaou method and studied by light microscopy In cases suspicious for craniopharyngioma fresh wet mounted preparations of cystic fluid were subjected to ordinary light microscopy for identification of cholesterol crystals [5 15]

Results

In all three cases of germinoma the CSF cytology was positive for malignant cells The overall cell morphology was that of an undifferentiated large cell carcinoma which in many respects would have been indistinguishable from a metastatic malignancy of this type since the cells bearing mostly an epithelium like morphology had an immature nucleus and a small to average amount of cytoplasm with no differentiating characteristics [11]

Some tumor cells arranged singly or in small groups were rounded and exhibited an active nucleus with a fine chromatin pattern and a prominent nucleolus and finely textured cytoplasm that was moderate in amount Such cells could have been derived from typical germinomatous (seminomatous) areas of the tumors (Figure 1)

The histology of these three tumors varied somewhat with the germinomatous component well defined in certain areas of the biopsy specimens while in other areas it was not as well defined In the latter areas they presented features of an undifferentiated large cell epithelial malignancy while retaining many of the features of the germinomatous tissue including a focal lymphocytic infiltration [13] It was not possible to use any type of lymphocytic component of the CSF specimens to support a specific diagnosis of germinoma for such exfoliated tumor tissue components were indistinguishable from reactive lymphocytic CSF elements [illegible] Interestingly case 3 had been diagnosed previously by tissue biopsy as a suprasellar craniopharyngioma and when the symptoms of a midline lesion recurred two years following radiotherapy neuroradiologic studies revealed an infiltrative lesion expanding posteriorly with positive CSF cytology characteristic of an atypical teratomatous (germinomatous) lesion Retrospective review of the previous biopsy disclosed that the suprasellar lesion consisted of an admixture of ill-defined germinoma with other malignant teratomatous tissues In this case it was difficult to determine by routine light microscopic study whether the malignant cells were derived from the germinomatous or other teratomatous elements

In the five craniopharyngiomas material obtained by aspiration of predominantly cystic lesions yielded squamous cells or anucleated squames with various degrees of keratinization occasional fragments of basaloid (ameloblastic type) epithelium abundant degenerative and histiocytic elements and cholesterol crystals the last were seen in two cases in wet mounted preparations (Figure 2)

In case 8 in which the cytologic diagnosis of craniopharyngioma was made the histology of a totally extirpated intrasellar cyst with suprasellar extension was consistent with an epithelial cyst of persistent Rathke s cleft origin lined partly by columnar and partly by squamous epithelium [11] The cytologic findings were those of other craniopharyngiomas with anucleated keratinized squames and abundant degenerative elements The aspiration cytology was unable to discriminate this benign cystic variant from ordinary craniopharyngiomas [illegible]

In the two cases of chromophobe adenoma aspiration cytology material was diagnostic In case 9 the presence of loose tissue fragments consisting of uniform tapering cells with focal palisading was indicative of a chromophobe adenoma with sinusoidal pattern and in case 10 papillary epithelium-type fragments with uniform cells provided the basis for a

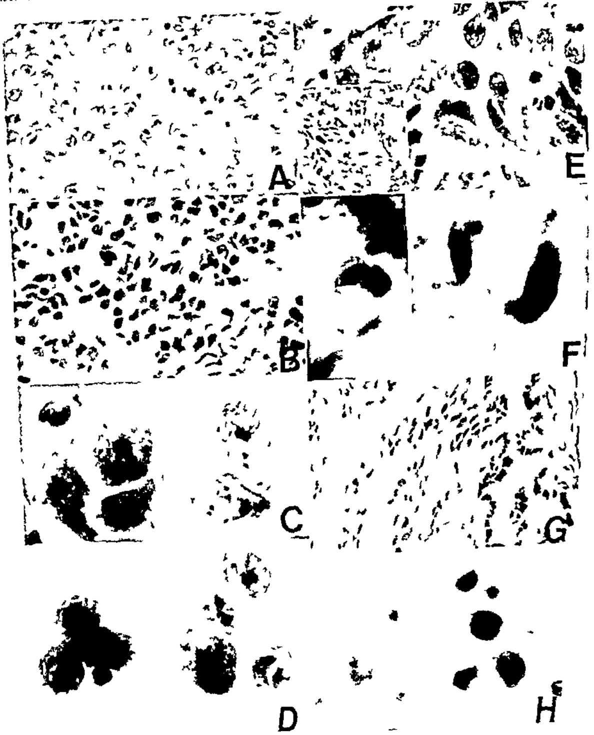


Figure 1
Germinomas. Case 1. (A) Histology. Area with seminomatous [illegible] ×400. [illegible] with lesser differentiation of tumor (hematoxylin and eosin ×400). (C, D) CSF [illegible] characters. Some cells in Figure 1C are reminiscent of seminoma cells [illegible] of lesser differentiation. Inset shows the seminoma p[illegible] [illegible] CSF cytology. Undifferentiated large cells (Pa[illegible] ×400). (G) Case [illegible] differentiation at left, embryonal glandular componen[illegible] undifferentiated tumor cells w[illegible] characters. [illegible] ×[illegible]

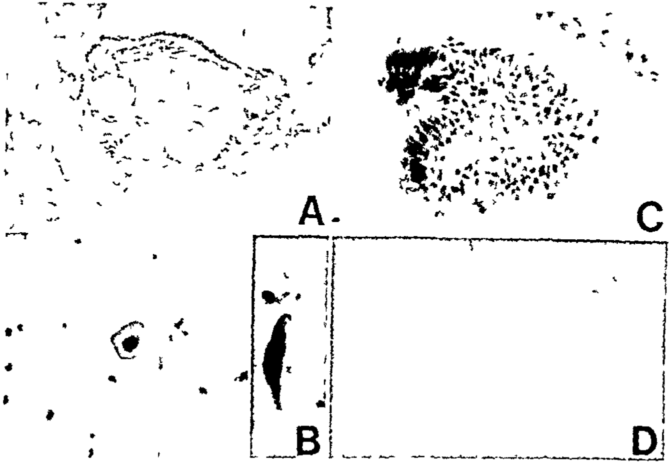


Figure 2
Craniopharyngioma (A) Histology Tissue nest with basaloid (ameloblastic type) elements at periphery and squamous elements with keratinization at center (hematoxylin and eosin × 40) (B) Aspiration cytology Anucleated and nucleated keratinized squamous cells (Papanicolaou stain × 250) (C) Aspiration cytology Basaloid (ameloblastic) epithelium (Papanicolaou stain × 400) (D) Cholesterol crystal (unstained wet mounted preparation × 100)

diagnosis of a chromophobe adenoma with papillary component (Figure 3)

Discussion

Primary intracranial midline tumors of the pineal pituitary axis although producing similarities in clinical and radiologic presentation are a nonhomogeneous group of neoplasms and the type of lesion determines to a great extent the diagnostic value of the cytologic material that can be used for its detection

CSF exfoliative cytology appears to be of value in the diagnosis of malignant teratomatous lesions (pineal or at times extrapineal) presenting very often with a germinomatous component for they are invasive and spreading tumors readily exfoliating and seeding in the subarachnoid linings [1,2,4,11] Yet their cytologic pattern in CSF is not well defined it is largely that of an undifferentiated large cell epithelial malignancy *The proper diagnosis is based to some* extent on the clinical and radiologic presentation along with the absence of a primary malignancy elsewhere in the body [4,11] It was not possible to utilize the lymphocytic component present for the diagnosis of a germinoma

CSF cytology was not performed in any of the cases of craniopharyngioma or pituitary adenoma These tumors are as a rule noninfiltrative and spontaneous exfoliation is uncommon Hence CSF cytology is usually of limited value in providing findings with definitive diagnostic morphology [1,7,8]

In addition in those reported cases of craniopharyngiomas with positive CSF cytology the changes described are not specific in cases of rupture of the capsule of these cystic tumors the elements escaping into the CSF are mostly reactive and of limited diagnostic value [3,14] Considering the above aspiration cytology remains the alternative method for a definitive cytologic diagnosis of this group of lesions

Yet even this method has its limitations when dis

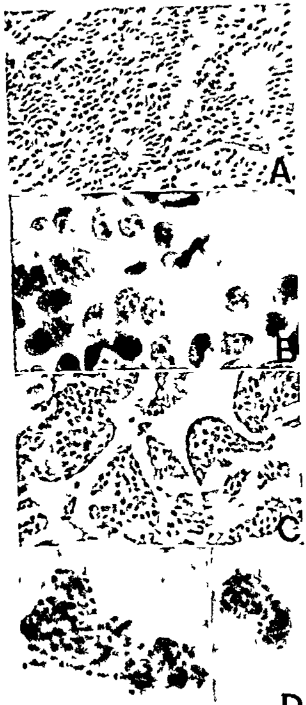


Figure 3
Chromophobe adenoma (A) Case 9 histology Sinusoidal pattern with perivascular arrangements of tapering cells (hematoxylin and eosin × 100) (B) Case 9 aspiration cytology Same cells focally palisading (Papanicolaou stain × 400) (C) Case 10 histology Uniform tissue with papillary like component (hematoxylin and eosin × 250) (D) Case 10 cytology Papillary tissue fragment with uniformity of cells (Papanicolaou stain × 250)

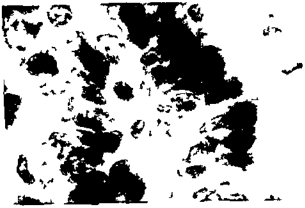


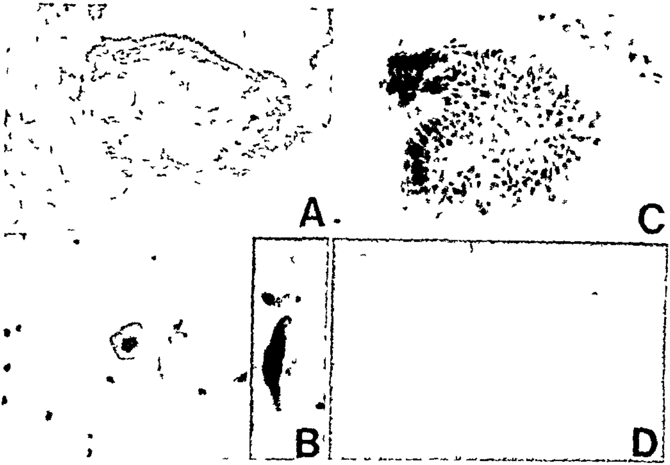
Figure 4
Posterior midline cystic lesion Aspiration cytology Atypical ependymal tissue Mitotic figure at center (Papanicolaou stain × 400)

tinguishing craniopharyngiomas from their cystic variants In such cases the exact clinical setting and results of improved neuroradiologic studies should be taken into consideration when interpreting the cytologic findings

The 11th case in this series was that of a 24 year old white female with a cystic lesion of the quadrigeminal plate producing posterior midline syndrome and obstructive hydrocephalus This by previous exploration was considered to be an arachnoidal cyst yet aspiration of the cyst contents at the time of reexploration produced what appeared to be atypical ependymal and/or choroid plexus elements with a low mitotic rate (Figure 4) [15] It was difficult to interpret the pathologic significance of these elements yet the prevailing opinion was that they were benign and in keeping with the clinical impression that they derived from a cystic lesion probably of an arachnoidal type which in some fashion had communicated with the ventricular system

A definitive diagnosis in this case has not been established but it serves to underscore the limitations of aspiration cytology in providing a conclusive diagnosis in cases of cystic midline brain lesions stressing also the need for a clinical and topographic correlation when interpreting the cytologic findings

This series does not include examination by cytologic methods of nerve tissue tumors of the midline structures Nevertheless the applicability of exfoliative CSF cytology for the diagnosis of gliomatous tumors is relatively limited and diagnostic aspiration methods appear to be more suitable



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Discussion

Primary intracranial midline tumors of the pineal pituitary axis although producing similarities in clinical and radiologic presentation are a nonhomogeneous group of neoplasms and the type of lesion determines to a great extent the diagnostic value of the cytologic material that can be used for its detection

CSF exfoliative cytology appears to be of value in the diagnosis of malignant teratomatous lesions (pineal or at times extrapineal) presenting very often with a germinomatous component for they are invasive and spreading tumors readily exfoliating and seeding in the subarachnoid linings [1,2,4,11] Yet their cytologic pattern in CSF is not well defined it is largely that of an undifferentiated large cell epithelial malignancy *The proper diagnosis is based to some extent on the clinical and radiologic presentation along with the absence of a primary malignancy elsewhere in the body* [4,12] *It was not possible to utilize the lymphocytic component present for the diagnosis of a germinoma*

CSF cytology was not performed in any of the cases of craniopharyngioma or pituitary adenoma These tumors are as a rule noninfiltrative and spontaneous exfoliation is uncommon Hence CSF cytology is usually of limited value in providing findings with definitive diagnostic morphology [1,7,9]

In addition in those reported cases of craniopharyngiomas with positive CSF cytology the changes described are not specific in cases of rupture of the capsule of these cystic tumors the elements escaping into the CSF are mostly reactive and of limited diagnostic value [3,14] Considering the above aspiration cytology remains the alternative method for a definitive cytologic diagnosis of this group of lesions

Yet even this method has its limitations when dis

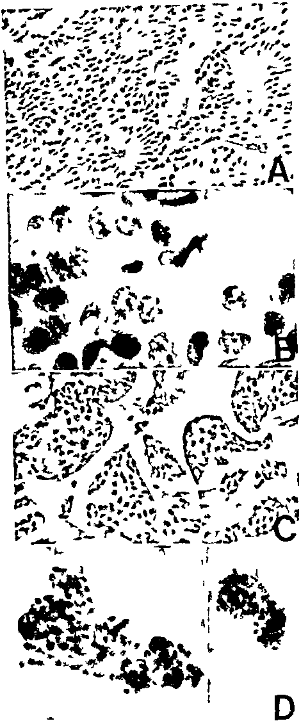

Figure 3
Chromophobe adenomas (A) Case 9 histology Sinusoidal pattern with perivascular arrangements of tapering cells (hematoxylin and eosin × 100) (B) Case 9 aspiration cytology Same cells focally palisading (Papanicolaou stain × 400) (C) Case 10 histology Uniform tissue with papillary like component (hematoxylin and eosin × 250) (D) Case 10 cytology Papillary tissue fragments with uniformity of cells (Papanicolaou stain × 250)

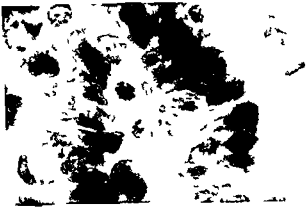

Figure 4
Posterior midline cystic lesion Aspiration cytology Atypical ependymal tissue Mitotic figure at center (Papanicolaou stain × 400)

tinguishing craniopharyngiomas from their cystic variants In such cases the exact clinical setting and results of improved neuroradiologic studies should be taken into consideration when interpreting the cytologic findings

The 11th case in this series was that of a 24 year old white female with a cystic lesion of the quadrigeminal plate producing posterior midline syndrome and obstructive hydrocephalus This by previous exploration was considered to be an arachnoidal cyst yet aspiration of the cyst contents at the time of reexploration produced what appeared to be atypical ependymal and/or choroid plexus elements with a low mitotic rate (Figure 4) [15] It was difficult to interpret the pathologic significance of these elements yet the prevailing opinion was that they were benign and in keeping with the clinical impression that they derived from a cystic lesion probably of an arachnoidal type which in some fashion had communicated with the ventricular system

A definitive diagnosis in this case has not been established but it serves to underscore the limitations of aspiration cytology in providing a conclusive diagnosis in cases of cystic midline brain lesions stressing also the need for a clinical and topographic correlation when interpreting the cytologic findings

This series does not include examination by cytologic methods of nerve tissue tumors of the midline structures Nevertheless the applicability of exfoliative CSF cytology for the diagnosis of gliomatous tumors is relatively limited and diagnostic aspiration methods appear to be more suitable

References

1 Den Hartog Jager WA Cytopathology of the cerebrospinal fluid examined with the sedimentation technique after Sayk J Neurol 9 155-177 1969

2 Jellinger K Primary intracranial germ cell tumors Acta Neuropathol 25 291 306 1973

3 Kline T Cytological examination of cerebrospinal fluid Cancer 15 591 597 1962

4 Kolmel HW Atlas of Cerebrospinal Fluid Cells Second edition New York Springer Verlag 1977 p 74

5 Koos WT Miller MH Intracranial Tumors of Infants and Children St Louis CV Mosby 1971 p 192

6 McCormack LJ Hazard JB Belovich D Gardner WJ Identification of neoplastic cells in cerebrospinal fluid by a wet film method Cancer 10 1293-1299 1957

7 Naylor B The cytologic diagnosis of cerebrospinal fluid Acta Cytol 8 141 149 1964

8 Oehmichen M Cerebrospinal Fluid Cytology An Introduction and Atlas Philadelphia WB Saunders 1976 p 155

9 Petito CK DeGirolami U Earle KM Craniopharyngiomas A clinical and pathological review Cancer 37 1944 1952 1976

10 Rubinstein LJ Tumors of the Central Nervous System Washington DC Armed Forces Institute of Pathology 1972 pp 269 284

11 Russell DC Rubinstein LJ Pathology of Tumors of the Nervous System Fourth edition Baltimore Williams & Wilkins 1977 pp 32 38 288

12 Takahashi M Color Atlas of Cancer Cytology Philadelphia JB Lippincott 1971 p 248

13 Teilum G Special Tumors of Ovary and Testis Philadelphia JB Lippincott 1976 p 380

14 Wertlake PT Markovits BA Stellar S Cytologic evaluation of spinal fluid with clinical and histologic correlation Acta Cytol 16 224 239 1972

15 Zulch KJ Brain Tumors Their Biology and Pathology Second edition New York Springer Publishing 1965 pp 69 93 231 260-263

Cytologic Evaluation of Ocular Lesions

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Evaluation was made of ocular cytologic specimens from 59 patients with clinically suspicious lesions 47 conjunctival scrapes 10 vitreous fluid aspirates 1 corneal scrape and 1 iris cyst aspirate Tissue correlation was available for 30 of the 59 cases A control group of 20 normal conjunctival scrapes was also evaluated

Cytomorphologic criteria for normal ocular specimens inflammatory lesions premalignant lesions and malignant lesions are presented These lesions included dysplasia (seven cases) carcinoma in situ *(four cases) squamous cell carcinoma (five cases) Meibomian gland carcinoma (three cases) basal cell carcinoma (two cases) metastatic adenocarcinoma (one case) melanoma (two cases) and retinoblastoma (one case) The remaining lesions were nonspecific inflammatory changes and normal variants The results of this study indicate that cytologic examination of ocular specimens can be a valuable adjunct in the workup of suspected neoplastic eye lesions*

The application of exfoliative cytology in the diagnosis of neoplastic or suspected neoplastic ocular lesions is still evolving To date few authors have described their experiences with ocular cytology[1 3 1] These studies have indicated that the diagnosis and clinical management of eye lesions are facilitated by cytologic evaluation of conjunctival scrapings or intraocular aspirations

This retrospective study was undertaken to evaluate the cytomorphologic criteria for normal ocular specimens and for benign and malignant lesions

Materials and Methods

Over a two year period ocular cellular samples from 59 patients with clinical ocular abnormalities treated at the Bascom Palmer Eye Institute Miami Florida were available for evaluation These included 11 cases of intraocular aspirations 47 cases of conjunctival scrapings and 1 corneal scraping An additional 20 conjunctival scrapings were obtained from normal volunteers as controls All of the patients were being evaluated for lesions in which the differential diagnosis included neoplastic disease Tissue biopsies were available for evaluation in 25 of 26 malignant lesions or their precursors 2 of 5 benign neoplasms and 3 of 28 nonneoplastic lesions

Conjunctival Specimens

Following adequate topical anesthesia with 0 5% proparacaine HCl the lesion was scraped with a cooled heat-sterilized platinum spatula The material obtained was smeared on a glass slide and quickly placed in 95% alcohol for fixation

Ocular Fluids

Ocular fluid specimens were obtained by the following procedure After general anesthesia an incision was made in the sclera and a small amount of vitreous fluid was allowed to herniate through the incision This fluid was then snipped off smeared on glass slides and immediately placed in 95% alcohol

Anterior chamber fluid was obtained after ade-

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quate retrobulbar anesthesia with 1% lidocaine HCl Using a 25-gauge needle and a 1-cc syringe a drop or two of material was obtained placed on a slide and fixed

Subretinal fluid was obtained after adequate anesthesia of the sclera and uvea A small incision was made in the sclera and the uvea was punctured Subretinal fluid was then collected smeared and fixed immediately

All specimens were stained with the Papanicolaou staining method Each cell sample was studied as to the presence of various normal and abnormal cells The number of cells their distribution cellular size and shape cytoplasmic and nuclear characteristics and the host response as reflected in the background of the smear as well as other findings were evaluated Whenever possible the cellular findings were correlated with tissue changes

Results

Ocular Fluid Specimens

As shown in Table I ocular fluid specimens from 11 patients including one anterior chamber aspirate one iris cyst aspirate and nine vitreous fluid aspirates were studied

The anterior chamber aspirate cell sample contained over 500 malignant cells derived from keratinizing squamous cell carcinoma The malignant squamous cells were approximately one third to one half the size of normal mature squamous cells and showed marked pleomorphism The nuclei were at least twice the size of the nuclei of mature squamous cells and were markedly hyperchromatic Nucleoli were inconspicuous

The iris cyst aspirate contained inflammatory cells with extracellular finely granular brown pigment consistent with melanin Tissue sections showed a typical epithelial iris cyst lined by benign stratified squamous epithelial cells

Vitreous fluid aspirates from nine patients included a case in which the cellular sample showed a small cell malignant tumor consistent with the diagnosis of retinoblastoma a case with abnormal cells of epithelial origin derived from an adenocarcinoma six cases with nonspecific inflammatory cells and one case in which the cell study was within normal limits All of these specimens contained pigment laden histiocytes probably retinal melanin pigment representing normal constituents

The vitreous aspirate cell sample from an 8 year-old girl with a clinical diagnosis of retinoblastoma contained malignant small cells approximately three to five times the size of neutrophils occurring singly and in loose aggregates An acinar pattern was not identified The cells were round or oval with markedly scanty cytoplasm and the hyperchromatic nuclei showed irregular distribution of granular chromatin material Micronucleoli were inconspicuous (Figure 1)

Table I *Intraocular Fluid Specimens by Cellular Interpretation*

	No of cases	Tissue confirmation
Squamous cell carcinoma	1	1
Small cell malignant tumor	1	0 (clinical retinoblastoma)
Adenocarcinoma	1	1
Inflammatory cells	7	1 (iris cyst)
No cellular elements	1	0
Total	11	3

The cell study from a patient with proven adenocarcinoma metastatic to the retina from the colon (Figure 2A) contained only 11 abnormal columnar cells occurring in three groupings (Figure 2B) The cytoplasm was finely vacuolated and moderate in amount Nuclei were eccentrically located with irregular distribution of finely granular chromatin Nucleoli were observed in 3 of the 11 abnormal cells

The remaining cellular samples from six patients contained inflammatory cells with no cellular evidence of malignancy or other atypical cells Subsequent clinical follow up of four cases revealed an inflammatory ocular process with no evidence of neoplasia In one case the cell sample revealed numerous polymorphonuclear leukocytes and few histiocytes A diagnosis of *Listeria monocytogenes* endophthalmitis was made when culture of vitreous fluid grew the specific organisms This case has been previously reported[1] In the remaining case the vitreous cell sample was obtained from a patient with a histologically confirmed reticulum cell sarcoma of the brain Occasional multinucleated giant cells were identified that may or may not have been related to previous therapy However no malignant cells were observed

Conjunctival Scrapes

Twenty conjunctival scrapes were obtained from ten volunteers with normal conjunctivas as controls These smears showed uniform low cuboidal cells occurring singly but predominantly in sheets with a nuclear-cytoplasmic ratio of 1 3 to 1 4 (Figure 3) The

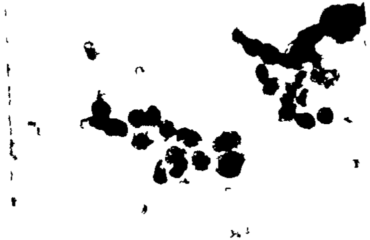


Figure 1
Malignant small cells from a vitreous aspirate of a g
retinoblastoma (× 640)

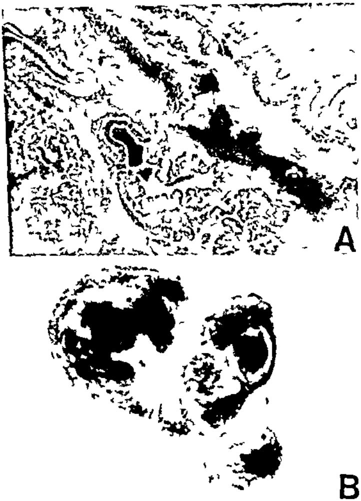


Figure 2
(A) Adenocarcinoma metastatic to the retina (× 12
group of abnormal columnar cells identified in a vit
aspirate from a patient with colonic adenocarcinom
static to the retina (× 640)

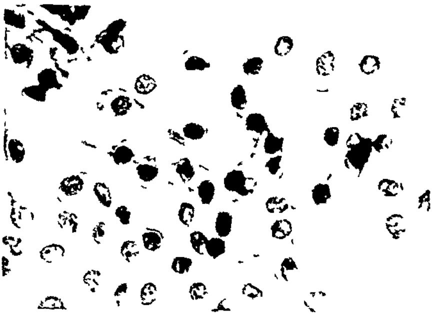


Figure 3
Normal cellular elements of a conjunctival scrape (X 640)

cytoplasm was cyanophilic to lightly eosinophilic *The nuclei were eccentrically located with uniformly* finely granular chromatin Occasionally goblet cells were present Specimens from black volunteers had cytoplasmic finely granular brown pigment

The cytologic findings of 47 patients with conjunctival lesions are listed in Table II The cell samples from two cases of squamous cell carcinoma of the conjunctiva proven by tissue biopsy contained over 600 malignant cells occurring singly and in aggregates consistent with a well-differentiated keratinizing squamous cell carcinoma (Figure 4)

Six cases of conjunctival squamous cell carcinoma *in situ* proven by tissue biopsy were reviewed (Figure 5A) The criteria for the diagnosis of squamous cell carcinoma *in situ* in the skin were utilized for the conjunctiva Of the six conjunctival scrapes five contained abnormal squamous cells and one was considered unsatisfactory due to lack of cellular material The findings in the five conjunctival scrapes were similar (Figure 5B) The number of abnormal cells varied from 50 to 300 The abnormal squamous cells were approximately one third to one half the size of normal mature squamous cells and were arranged predominantly in aggregates They appeared polygonal or oval The cytoplasm was moderately abundant and stained cyanophilic and orangeophilia was less conspicuous The centrally placed round to oval hyperchromatic nuclei occupied approximately 40% to 60% of the cells with uniformly distributed granular chromatin Nucleoli were observed in less than 10% of the abnormal cells Pyknosis was inconspicuous Hyperkeratosis was evident in four of the five specimens The background of the smear was clean

Table II Conjunctival Scrapes by Cellular Interpretation

	No of cases	Tissue confirmation
Poorly differentiated carcinoma	3	3 (Meibomian (cancer)
Squamous cell carcinoma	2	2
Basal cell carcinoma	2	2
Malignant melanoma	2	2
Squamous *in situ* cancer	5	5
Squamous dysplasia	8	8
Atypical nevus cells	3	1 (adolescent type)
Nevus cells	2	1 (adult type)
Hyperkeratosis	2	2 (1 pinguecula 1 corneal opacity)
Herpetic conjunctivitis	1	—
Normal (few inflammatory cells)	16	—
Unsatisfactory	1	1 (carcinoma *in situ*)
Total	47	27

Conjunctival scrapes from eight cases of histologically confirmed squamous dysplasia were studied Histologically there was hyperplasia of the squamous epithelium with slight abnormal differentiation reminiscent of a mild dysplasia of the uterine cervix (Figure 6A) The morphologic changes of dysplasia as defined were much less severe than those of squamous cell carcinoma *in situ* These differences were also reflected in the cellular samples One of the eight cell samples was considered unsatisfactory due

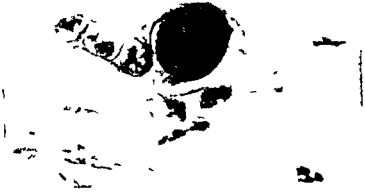


Figure 4
Cellular changes of squamous cell carcinoma of the conjunctiva (× 640)

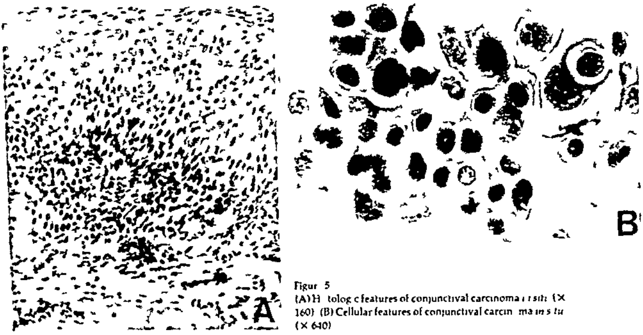


Figure 5
(A) Histologic features of conjunctival carcinoma *in situ* (× 160) (B) Cellular features of conjunctival carcinoma *in situ* (× 640)

to a lack of cellular material the remaining seven contained atypical squamous cells The number of atypical squamous cells varied from 15 to 20 per specimen and they were large approaching the size of normal mature squamous cells (Figure 6B) The cytoplasm was abundant and stained eosinophilic or orangeophilic The hyperchromatic nuclei were centrally placed and enlarged to twice the size of the nucleus of a normal mature squamous cell The chromatin was uniformly finely granular Nucleoli were not observed Mild hyperkeratosis was evident in cell samples There was no tumor diathesis

Conjunctival cell samples from three cases of histologically confirmed Meibomian gland carcinoma were evaluated and the cytologic changes correlated with those seen in the tissue specimens (Figure 7A) The cellular features from these three cases were similar the more than 500 malignant cells per sample had

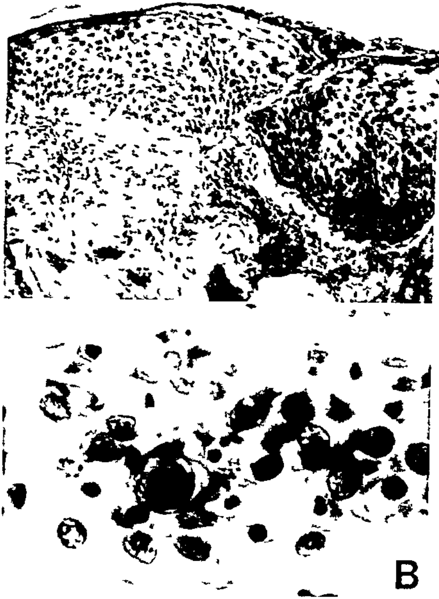


Figure 6
(A) Histologic features of conjunctival dysplasia (× 160)
(B) Cellular features of conjunctival dysplasia (× 640)

the features of a poorly differentiated adenocarcinoma The oval to low cuboidal malignant cells were two to three times the size of normal low cuboidal conjunctival cells and occurred singly and in irregular sheetlike aggregates (Figure 7B) The cytoplasm was cyanophilic finely granular or vacuolated The enlarged hyperchromatic round nuclei were usually eccentrically located within the cells and occupied up to 60% to 80% of the cell area The finely granular chromatin was irregularly distributed and nucleoli were encountered frequently The background had a watery granular appearance with lysed red blood cells inflammatory cells and cellular detritus constituting a tumor diathesis

Conjunctival cell samples from two cases of histologically confirmed malignant melanoma revealed numerous isolated and loose syncytial aggregates of oval or polygonal malignant melanoma cells each measuring approximately one-quarter to one third the size of normal squamous cells Cytoplasm was moderately abundant granular and stained cyanophilic Brown to black pigment of varying sizes in the cytoplasm was identified in many of these malignant cells (Figure 8) The large round nuclei occupying up to 50% of the cells were located slightly eccentrically There was irregular distribution of the granular chromatin material Single or multiple macronucleoli often irregular in shape were conspicuous Binucleation of the malignant cells was also encountered frequently Extracellular brown pigment associated with a tumor diathesis was also evident

Conjunctival scrapes from three adolescent patients with nevi revealed an admixture of normal low cuboidal cells squamous metaplastic cells and nevus cells The number of nevus cells in conjunctival scrapes was less than that obtained from malignant

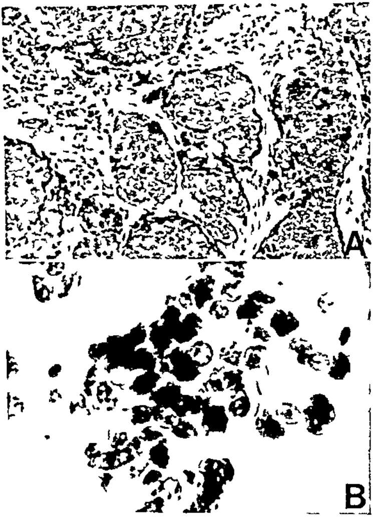


Figure 7
(A) Histologic features of Meibomian gland carcinoma (× 160) (B) Malignant cells derived from Meibomian gland carcinoma (× 640)

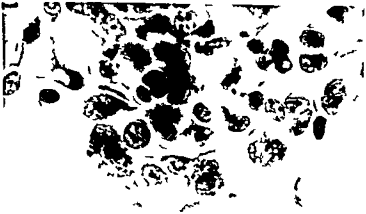
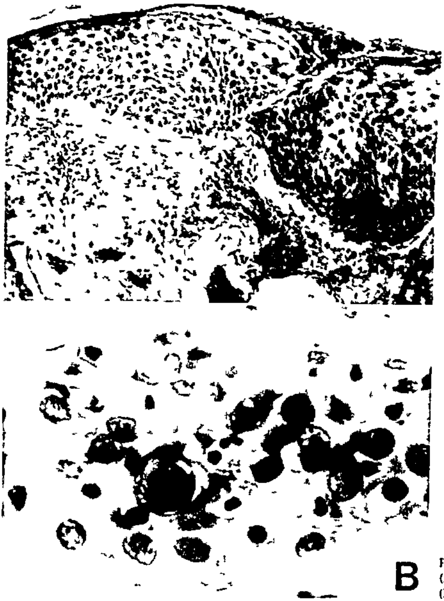


Figure 8
Cellular changes of malignant melanoma of the conjunctiva. Note cytoplasmic pigments (× 640)



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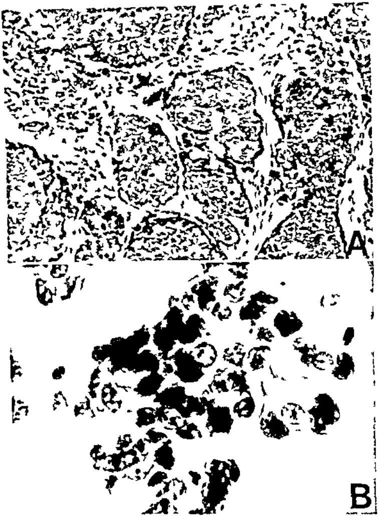

Figure 7
(A) Histologic features of Meibomian gland carcinoma (× 160) (B) Malignant cells derived from Meibomian gland carcinoma (× 640)

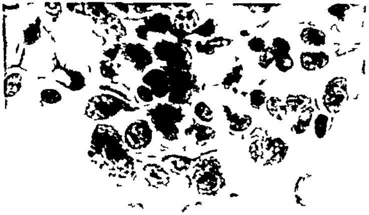

Figure 8
Cellular changes of malignant melanoma of the conjunctiva Note cytoplasmic pigments (× 640)

melanoma The nevus cells occurred in loose aggregates and isolated cells were unusual (Figure 9) They were approximately half the size of malignant melanoma cells The nevus cells appeared oval or spindled with a moderate amount of cyanophilic cytoplasm Although the oval nuclei occupied up to 50% of the cells they were one half the size of their malignant counterparts The chromatin was uniformly finely granular Single and multiple micronucleoli were encountered frequently Cytoplasmic brown pigment was inconspicuous The smears lacked a tumor diathesis The presence of enlarged nuclei and nucleoli reflected cellular activity commonly encountered in nevi of adolescents In contrast conjunctival scrapes of nevi from two adult patients revealed similar nevus cells in which the nuclei were smaller than those derived from nevi of adolescents and the micronucleoli tended to be single and observed infrequently (Figure 10)

The conjunctival scrapes from two patients with histologically confirmed ulcerated basal cell carcinoma were characterized by relatively uniform abnormal small cells occurring predominantly in aggregates and infrequently isolated (Figure 11) The cyanophilic cytoplasm was scanty and the round nuclei contained irregularly finely granular chromatin Micronucleoli were observed infrequently A tumor diathesis was observed in both specimens

Typical multinucleated giant cells with ground glass nuclei and chromatinic thickening of the

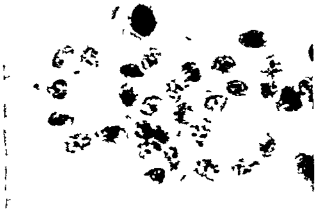

Figure 9
Atypical nevus cells from a juvenile nevus of the conjunctiva (× 640)

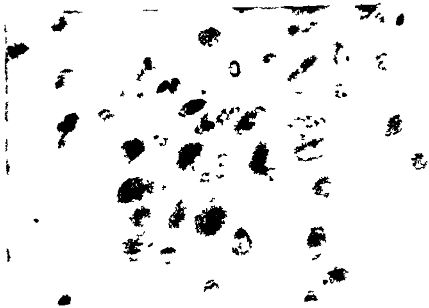

Figure 10
Nevus cells from a conjunctival nevus of an adult (× 640)

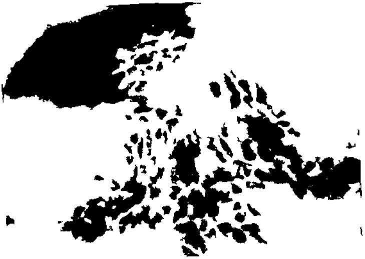

Figure 11
Cellular changes consistent with basal cell carcinoma of the conjunctiva (× 640)

nuclear membrane were observed in the conjunctival cell sample obtained from a patient with clinical evidence of herpes conjunctivitis

The conjunctival sample of one patient who presented with clinical findings typical of conjunctival pinguecula showed superficial squamous cells and hyperkeratosis

Corneal scrape from a case of clinically suspicious squamous cell carcinoma revealed limited hyperkeratosis and superficial squamous cells consistent with the histologic findings which also showed squamous epithelium with hyperkeratosis

Cell samples from the remaining 16 conjunctival scrapes showed normal low cuboidal cells with occasional inflammatory elements and no atypical cells The subsequent clinical course was uneventful in these cases

Discussion

Examination of eye cytology specimens for infectious diseases and other inflammatory lesions has long been recognized by ophthalmologists as a useful tool in diagnosis and management [1,3,[illegible],13,[illegible]] Only recently have ocular specimens been applied for the evaluation of malignant and premalignant lesions

The use of intraocular aspirations for the diagnosis of retinoblastoma has been reported [illegible] The cytologic findings in our case were similar to those described previously although typical rosette formations were not evident in our case Intraocular extension of squamous cell carcinoma of the conjunctiva has been described previously [10] Examination of the aqueous humor is a useful technique for diagnosing this unusual route of tumor extension

Other tumors reported in the literature that have been diagnosed by intraocular aspiration include malignant melanoma [8] malignant lymphoma [9] orbital extension of a maxillary sinus transitional cell carcinoma[9] and juvenile xanthogranuloma [12] This study adds a case of metastatic adenocarcinoma of the colon to this list

Inflammatory cells that phagocytize melanin pigment and occasionally benign melanin pigment containing retinal cells found in the fluid aspirate from nontumoral retinal detachment can be confused with malignant melanoma However these benign pigmented cells lack the typical malignant criteria for malignant melanoma of the retina [illegible]

The normal cytology of the conjunctiva and cornea has been described previously [1,3,[illegible],14] It is important to realize that the conjunctiva is composed of cuboidal to columnar cells with interspersed goblet cells Any squamous differentiation or keratin production is abnormal Squamous differentiation or hyperkeratosis of the conjunctiva may be the result of papilloma chronic irritation vitamin A deficiency keratoconjunctivitis sicca or premalignant atypia In this study hyperkeratosis was associated with pinguecula nevi dysplasia carcinoma *in situ* squamous cell carcinoma and basal cell carcinoma Often it is the only clue to an underlying surface abnormality

The cellular features of conjunctival squamous cell carcinoma and basal cell carcinoma observed in this study were similar to those previously described [illegible] However cytologic and histopathologic characteris tics of squamous carcinoma *in situ* and dysplasia of the conjunctiva are less well documented The cellular features of squamous cell carcinoma *in situ* observed in this study were similar to Bowen s disease or squamous cell carcinoma *in situ* observed in epidermoid tissues elsewhere in the body The dysplastic cells resembled a mild keratinizing dysplasia observed in the cervix

This report appears to be the first to describe the cytologic characteristics of Meibomian gland carcinoma from a conjunctival scrape Meibomian gland carcinoma is a slow-growing yellow-white tumor in the tarsal portion of the lid As the tumor grows it erodes the lid margin or conjunctiva to produce a nodular or lobulated mass

The histologic pattern of malignant melanoma of the conjunctiva has been described [2] The cellular features *of malignant melanoma from conjunctival* scrapes are similar to those reported in intraocular melanoma and from other body sites [3,8] Adolescent *conjunctival and skin nevi usually grow until puberty* or late teens and then tend to regress Consequently nevus cells obtained from adolescent patients tend to *appear more reactive* as reflected by the presence of nuclear enlargement and nucleoli The presence of both these features may be confused with cells derived from malignant melanoma

Conjunctival and corneal cytologic smears can be obtained by a simple office procedure using local anesthesia and equipment readily available to ophthalmologists When indicated multiple sites can be sampled Intraocular aspirations are less difficult to obtain and probably more efficient for evaluation when compared with intraocular biopsies [12] Ophthalmologists at our institution state that complications are minimal if this technique is applied properly The eyes replace the removed fluid very rapidly

This study supports reports by others that cytologic evaluation of intraocular fluid[1,7,11,13] and conjunctival[3,4,8,14,15,16] or corneal scrapes[8,9] is *a helpful* procedure both in the outpatient clinic and for hospitalized patients in the diagnosis and management of *ocular disease In our study because of the close* cooperation between the clinician and the cytology laboratory problems relating to sampling techniques fixation and processing of cytologic specimens were eliminated resulting in optimum cellular preparations for accurate evaluation Only 1 of 1 cytologic specimens from 59 patients and 20 norm controls was considered unsatisfactory due to lack o cellular material In the future ophthalmologis probably will become accustomed to the practice o needle aspirations or conjunctival and corneal scraping in the evaluation of premalignant and malignan lesions as well as infectious lesions

References

1 Abbott RL Forster RK Rebell G *Listeria monocytogenes en*dophthalmitis with a black hypopyon *Am J Ophthalmol 8* 715-719 1978

2 Bernardino V Naidoff M Clark W Malignant melanomas o the conjunctiva Am J Ophthalmol 82 383-394 1976

3 Duszynski L Cytology of the conjunctival sac Am J Ophthalmol 37 576-580 1954

4 Dykstra PC Dykstra BA The cytologic diagnosis of carcinoma and related lesions of the ocular conjunctiva and cornea J Am Acad Ophthalmol Otolaryngol 73 [illegible] 1969

5 Hauser SA Moore NA Suckle AW Cytology of allergic con*junctivitis Arch Ophthalmol 11 1037 1053* 1973

6 Kimura S Thygeson PL The cytology of external disease Am J Ophthalmol 39 137 145 1955

7 Lam KW Constable IJ Schepens CL Subretinal fluid Loenzymes and cytologic studies Invest Ophthalmol 11 1037 1053 1973

8 Naib ZM Cytology of ocular lesions Acta Cytol 16 178-185 1972

9 Naib ZM Clepper AS Elliott SR Exfoliative cytology as an aid in the diagnosis of ophthalmic lesions Acta Cytol 11 295-303 1967

10 Nicholson DH Herschler J Intraocular extension of squamous cell carcinoma of the conjunctiva Arch Ophthalmol 95 843-846 1977

11 Rodriguez A Diagnosis of retinoblastoma by cytology examination of the aqueous and the vitreous Mod Probl Ophthalmol 18 142 149 1977

12 Sagiroglu N *Özgonul T Muderris S Diagnostic intraocular* cytology Acta Cytol 19 32 37 1975

13 Shields JA Lerner HA Felberg NT Aqueous cytology and enzymes in nematode endophthalmitis Am J Ophthalmol 84 319 322 1977

14 Thatcher RW Darougar S Jones B Conjunctival impression cytology Arch Ophthalmol 95 678-681 1977

15 Thelmo W Coordas J Davis P Marshall K The cytology of acute bacterial and follicular conjunctivitis Acta Cytol 16 172 177 1972

16 Thygeson P The cytology of conjunctival exudates Am J Ophthalmol 29 1499 1512 1946

Cytopathology of Pericardial Effusions

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Steven I Hajdu M D F I A C
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During a period of four years 158 cytologic specimens obtained from pericardial effusions of 120 patients were examined Malignant neoplastic cells were identified in 90 (57%) specimens from 72 (60%) patients Eighty percent of the neoplasms were epithelial and 20% were nonepithelial in origin The most common epithelial tumors were pulmonary (56%) and mammary (39%) neoplasms and most were adenocarcinomas In general the cytologic smears were moderately cellular with blood and occasional atypical mesothelial cells in the background There were few reactive cells such as histiocytes and lymphocytes in cytologically positive specimens

Pleomorphic nuclei and prominent nucleoli were dominant features of pulmonary adenocarcinomas Tumor cells of mammary carcinoma were more uniform and often formed cell balls Epidermoid carcinomas were characteristically poorly differentiated having a striking resemblance to adenocarcinoma In most instances several months elapsed between initial diagnosis of the primary neoplasm and a positive pericardial cytologic finding The majority of the patients died within ten months after malignant tumor cells were recovered from pericardial effusion Because of the serious clinical implications a cytologic diagnosis of cancer in pericardial effusions must never be based on scanty or equivocal evidence If necessary any doubt should be resolved by examination of additional material

Metastasis to the heart is not uncommon in patients with advanced neoplasms [1 9 13] Bisel et al [1] on review of 500 consecutive autopsies at Memorial Hospital noted a prevalence of 21% In another study Scott and Garvin[13] found that the heart parietal pericardium or both were involved in 10 9% of 1 082 autopsied patients with malignant neoplasms

Pericardial effusion in patients with cancer does not necessarily denote metastasis to the heart and cytologic examination of the fluid is helpful in the differential diagnosis For the patient with neoplastic cardiac tamponade cytology can provide an immediate and accurate diagnosis in a life threatening emergency [14] Yet although pericardiocentesis has been used in medical practice for over 100 years [7] there are few studies[2 3 5 8 12 15] of the cytology of pericardial effusions None is based on a large series of cases

Materials and Methods

In a period of four years (1974–78) 158 specimens of pericardial fluid were examined by the Cytology Service of the Memorial Sloan Kettering Cancer Center The specimens were collected from 120 patients by pericardiocentesis and submitted to the laboratory fixed with equal amounts of 50% ethyl alcohol After centrifugation the supernatant was decanted and the sediment was transferred to a prealbuminized glass slide by means of a curette type spoon With a clean glass slide the specimen was crushed and spread using a rotary motion until a smooth sandy surface was obtained The smears were fixed immediately in 95% ethyl alcohol for 20 minutes and then stained by the Papanicolaou method The rest of the sediment if any was fixed in 10% formaldehyde processed as a cell block according to routine histologic techniques and stained with hematoxylin and eosin In this study for the sake of simplicity cytologic specimens originally interpreted either as nega

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tive or atypical are grouped together and referred to as *negative* all of the cases that were diagnosed cytologically as suspicious or positive for malignant cells are tabulated as *positive*

Results

One hundred fifty-eight specimens obtained from pericardial effusions of 120 patients were examined (Table I) The age of the patients ranged from 8 to 84 years with most between 40 and 70 (Figure 1) There were 68 men and 52 women Ninety (57%) specimens from 72 (60%) patients were interpreted as either positive or suspicious for malignant cells Eighty percent of the tumors were epithelial and 20% were nonepithelial in origin (Table II) The primary sites of 71 tumors metastatic to the pericardium are illustrated in Figure 2 One of the cases originally reported as isolated suspicious cells " and on review considered mesothelial atypia only, later proved to be a case of idiopathic hypertrophic subaortic stenosis with pericardial effusion Fifty three cytologic specimens from 48 patients were interpreted as negative

Epithelial Neoplasms

There were 71 (79%) specimens from 57 (80%) patients (Table III) The most common epithelial neoplasms were pulmonary (56%) and mammary (39%)

Table I *Cytopathology of Pericardial Effusions (1974 '78)*

	Number of patients	Number of specimens	
		Positive	Negative
Positive cases	72	90	15
Negative cases	48	—	53
Total	120	90	68

Table II *Histologic Type of Primary Neoplasms Metastatic to the Pericardium*

	Number (%)
Epithelial neoplasms	57 (80%)
Adenocarcinoma	44 (62%)
Epidermoid carcinoma	7 (10%)
Miscellaneous	6 (8%)
Nonepithelial neoplasms	14 (20%)
Mesothelioma	4 (6%)
Lymphoma and leukemia	3 (4%)
Miscellaneous	7 (10%)
Total	71 (100%)

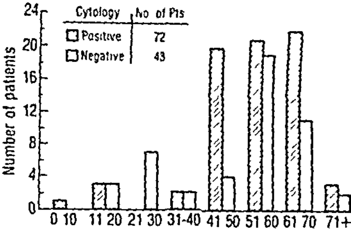

Figure 1
Age distribution of 120 patients with pericardial effusions

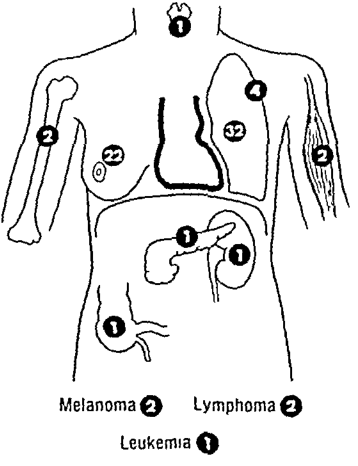

Figure 2
The primary sites of 17 tumors metastatic to the pericardium One of the two melanomas was a left leg primary In the other case the primary site was not identified One of the two patients with malignant lymphoma had lymphocytic lymphoma and the other patient had widespread Hodgkin s disease

carcinomas and most were adenocarcinomas (77%) These were followed in frequency by epidermoid carcinoma (12%) oat cell carcinoma (5%) and other less common neoplasms (6%)

Table III *Histologic Type and Anatomic Site of the Primary Neoplasms with Pericardial Effusion*

	No of patients		No of specimens	
	Total	Positives	Total	Positives
Epithelial neoplasms				
Lung	56	32	69	40
Breast	25	22	36	28
Gastrointestinal tract	3	1	3	1
Miscellaneous	5	2	5	2
Nonepithelial neoplasms				
Lymphoma and leukemia	8	3	13	4
Germ cell tumor	5	0	6	0
Mesothelioma	4	4	5	4
Miscellaneous	13	7	18	10
Total	119	71	155	89

Table IV *Clinical Significance of Positive Cytologic Findings*

	Average interval between 1st tissue diagnosis and positive pericardial cytology (months)	Average length of follow up (months)
Epithelial neoplasms		
Lung	6	5
Breast	48	10
Miscellaneous	74	3
Nonepithelial neoplasms		
Mesothelioma	18	7
Lymphoma and leukemia	63	3
Miscellaneous	19	2

There were 40 pericardial samples from 32 patients with lung cancer who had positive pericardial cytology The majority (21) of the lung pulmonary neoplasms were adenocarcinomas followed by epidermoid carcinoma (6) oat cell carcinoma (3) undifferentiated large cell carcinoma (1) and carcinoid tumor (1) The interval between the first tissue diagnosis of the pulmonary tumor and the positive pericardial cytology ranged from 0 to 21 months with an average of 6 months (Table IV) In six patients the only cytologic diagnosis of cancer was made on pericardial fluid Twenty patients had earlier cytologic specimens with cancer from 6 days to 16 months (average 5 months) prior to the positive pericardia cytology Most of the specimens 15 of 25 were tracheobronchial in origin Five patients had late cytologic specimens with cancer from 3 to 39 day (average 11 days) after the positive pericardial cytology (Table V) Most of the specimens four of five *were pleural effusions Ninety percent of the patient* who had cytologically positive pericardial effusion in association with lung carcinoma died within fiv months of cytologic diagnosis

There were 28 pericardial specimens from 22 patients with mammary carcinoma who had positiv pericardial cytology The interval between the firs tissue diagnosis of mammary carcinoma and th positive pericardial cytology ranged from 0 to 24 months with an average of 48 months (Table IV) Positive pericardial cytology was the only cytologi *finding of* cancer in ten patients (45%) Six patient had positive cytology in other specimens from days to 4 months (average 26 days) prior to the pos tive pericardial cytology (Table V) Most of th specimens six of eight were pleural effusions Seve patients had positive cytologic findings in othe specimens from 8 days to 12 months (average months) after the positive pericardial cytology Mo

Table V *Associated Positive Cytologic Findings at Other Sites*

	Total no of patients with positive pericardial cytology	Number of patients with positive cytology in other specimer	
		Prior to positive pericardial cytology	Subsequent to positive pericardial cytology
Epithelial neoplasms			
Lung	32	20	5
Breast	[illegible]	6	7
Miscellaneous	3	1	1
Nonepithelial neoplasms			
Mesothelioma	4	3	1
Lymphoma and leukemia	3	0	2
Miscellaneous	7	2	1
Total	71	3	17

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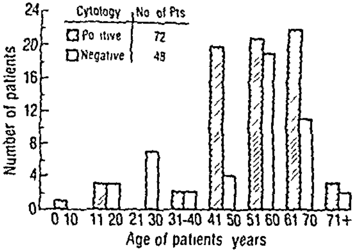

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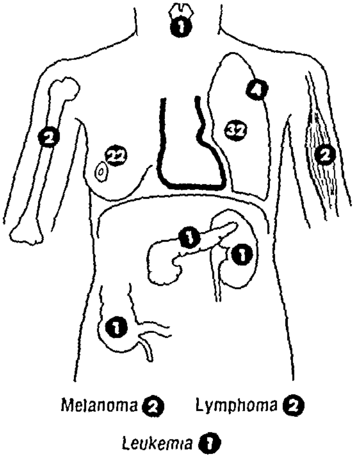

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There were 28 pericardial specimens from 22 patients with mammary carcinoma who had positive pericardial cytology The interval between the first tissue diagnosis of mammary carcinoma and the positive pericardial cytology ranged from 0 to 240 months with an average of 48 months (Table IV) Positive pericardial cytology was the only cytologic finding of cancer in ten patients (45%) Six patients had positive cytology in other specimens from 2 days to 4 months (average 26 days) prior to the positive pericardial cytology (Table V) Most of the specimens six of eight were pleural effusions Seven patients had positive cytologic findings in other specimens from 8 days to 12 months (average 5 months) after the positive pericardial cytology Most

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	Average interval between 1st tissue diagnosis and positive pericardial cytology (months)	Average length of follow up (months)
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	Total no of patients with positive pericardial cytology	Number of patients with positive cytology in other specimens	
		Prior to positive pericardial cytology	Subsequent to positive pericardial cytology
Epithelial neoplasms			
Lung	3	20	5
Breast	22	6	7
Miscellaneous	3	1	1
Nonepithelial neoplasms			
Mesothelioma	4	3	1
Lymphoma and leukemia	3	0	2
Miscellaneous	7	2	1
Total	71	32	17

of these specimens six of seven, were pleural effusions Nineteen of the 22 patients who had cytologically positive pericardial effusion in association with mammary carcinoma died within ten months of cytologic diagnosis

There were two other adenocarcinomas with cytologically positive pericardial effusion one spindle and giant cell carcinoma of the pancreas and one adenocarcinoma of the colon Both cases showed features of adenocarcinoma cytologically

Most of the specimens collected from patients who had pulmonary adenocarcinoma were moderately to highly cellular Only 4 of 25 specimens contained few malignant or suspicious cells The neoplastic cells were medium to large with much variation in size and shape and were isolated or arranged in clusters of often overlapping cells Multinucleated malignant tumor cells were not uncommon (Figures 3A and 3B) 'Indian file' arrangements and acinus formation were observed occasionally (Figures 3A and 4A) Nuclei were single or multiple round or ovoid and mostly irregular with marked variation in size and shape Bizarre appearing nuclei were commonly seen The nuclear membrane was well defined rather thick and often very irregular or lobulated Nuclear chromatin was hyperchromatic and coarse or vesicular Prominent nucleoli occurred in most of the nuclei Cytoplasm was moderate to abundant basophilic and finely vacuolated The cytoplasmic membrane was well defined in the majority of the cells Some of the tumor cells had single or multiple large vacuoles in the cytoplasm with occasional signet ring cells The background of most of the specimens showed blood and mild to moderate mesothelial atypia with occasional papillary mesothelial cell formations Some of the specimens showed a few lymphocytes and/or histiocytes in the background

The pericardial specimens collected from patients who had mammary carcinoma were moderately to highly cellular Only 1 of 28 specimens had few suspicious cells The neoplastic cells were medium in size and rather uniform Most were isolated though they had a tendency to form small clusters cords or cell balls that contained a few to several nuclei (Figures 5A to 5D) Indian file arrangements were noted (Figure 4C) Multinucleated tumor cells were rare Nuclei

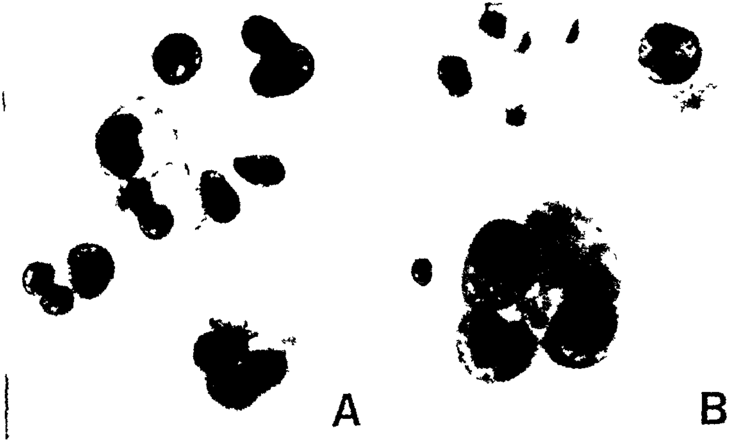


Figure 3
Metastatic pulmonary adenocarcinoma pericardial effusion (A) Tumor cells show multinucleation and acinus formation Note the pleomorphism of the nuclei and prominence of the nucleoli (Papanicolaou × 570) (B) Tumor cells are present both singly and in clusters Note the overlapping of the nuclei in clusters (Papanicolaou stain × 570)

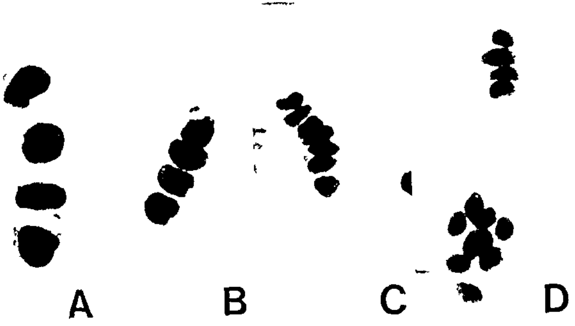


Figure 4
Indian file arrangements in pericardial effusions (Papanicolaou stain × 500) (A) Metastatic pulmonary adenocarcinoma (B) Oat cell carcinoma (C) Mammary carcinoma (D) Carcinoid tumor

were round or ovoid with only slight variation in size and shape The nuclear membrane of the cells in organoid groups were flattened or molded against each other Nuclei were hyperchromatic with coarse clumps of chromatin Nucleoli were present in most but not all of the neoplastic cells Cytoplasm was scanty to moderate basophilic and in some cells finely vacuolated In a small percentage of cases the tumor cells of metastatic mammary carcinoma bore a striking resemblance to those of adenocarcinoma of the lung in pericardial effusions (Figures 5C to 5G) The nuclei of these cells were single or multiple with marked variation in size and shape Many tumor cells had large cytoplasmic vacuoles Most specimens contained blood with mild to moderate mesothelial atypia and a moderate number of lymphocytes and/or histiocytes in the background

All of the epidermoid carcinomas with the exception of a laryngeal carcinoma were poorly differentiated and difficult to distinguish from adenocarcinoma cytologically (Figures 6A to 6C) However there were minor differences The cells of epidermoid carcinoma had a lesser tendency to form clusters though they were occasionally arranged in sheets which were more readily seen in cell blocks (Figure 6C) Nuclei were more hyperchromatic with a more even distribution of chromatin and less nuclear clearing Interestingly nucleoli were often quite prominent Cytoplasm was slightly denser than for adenocarcinoma and contained no vacuoles Cytoplasmic orangeophilia was not observed in these cases The background of the majority of specimens showed blood with mild to moderate mesothelial atypia including some papillary arrangements of mesothelial cells Reactive cells such as lymphocytes and histiocytes were relatively rare

Pericardial specimens from patients who had oat cell carcinoma of the lung were all highly cellular The tumor cells were small with occasional medium sized mononucleated cells The majority of the tumor cells were isolated but cluster and cord formation did occur and cell in-cell arrangements were occasionally seen (Figure 4B) Nuclei were single round or ovoid with variation in size and shape The nuclear membrane was well defined rather thick and irregular The nuclear membrane of cells in clusters or cords was flattened or molded nuclei were hyperchromatic and chromatin was coarsely clumped Nucleoli were present in many of the tumor cells Cytoplasm was scanty to moderate and usually well defined The background of the smears showed blood with occasional atypical mesothelial cells Re

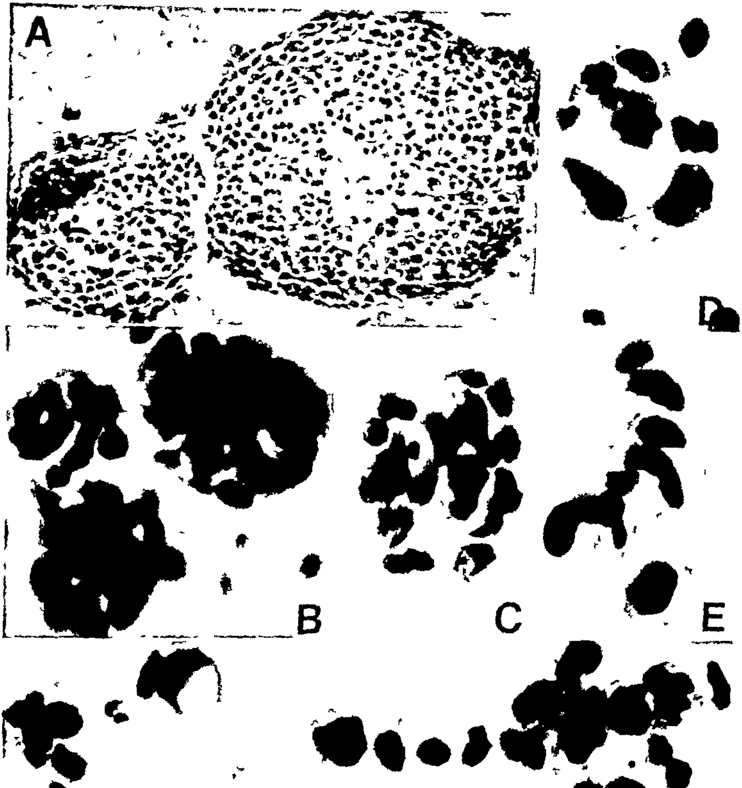


Figure 5
Metastatic mammary carcinoma, pericardial effusion. (A) Tendency to form cell balls and uniformity of the tumor cells are dominant features of metastatic mammary carcinoma (cell block, hematoxylin and eosin, × 140). (B) The same features shown in Figure 5A are evident in a smear (Papanicolaou stain, × 570). (C–G) The nuclei of metastatic mammary carcinoma appear pleomorphic, and the cytoplasm contains large vacuoles. Note the striking resemblance to metastatic pulmonary adenocarcinoma illustrated in Figure 3 (Papanicolaou stain, × 570).

active cells such as lymphocytes and histiocytes were rare

There was one case of metastatic undifferentiated large cell carcinoma of the lung The specimen was very cellular and the neoplastic cells bore a striking resemblance to those of epidermoid carcinoma There also was a case of metastatic carcinoid tumor of the lung Tumor cells in this case had a tendency to form loose groupings and occasional Indian file arrangements The nuclei were round or oval and were hyperchromatic (Figure 4D)

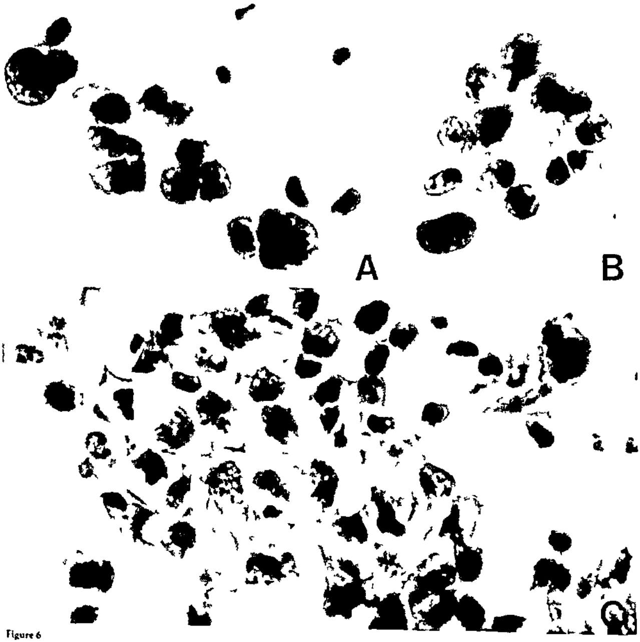
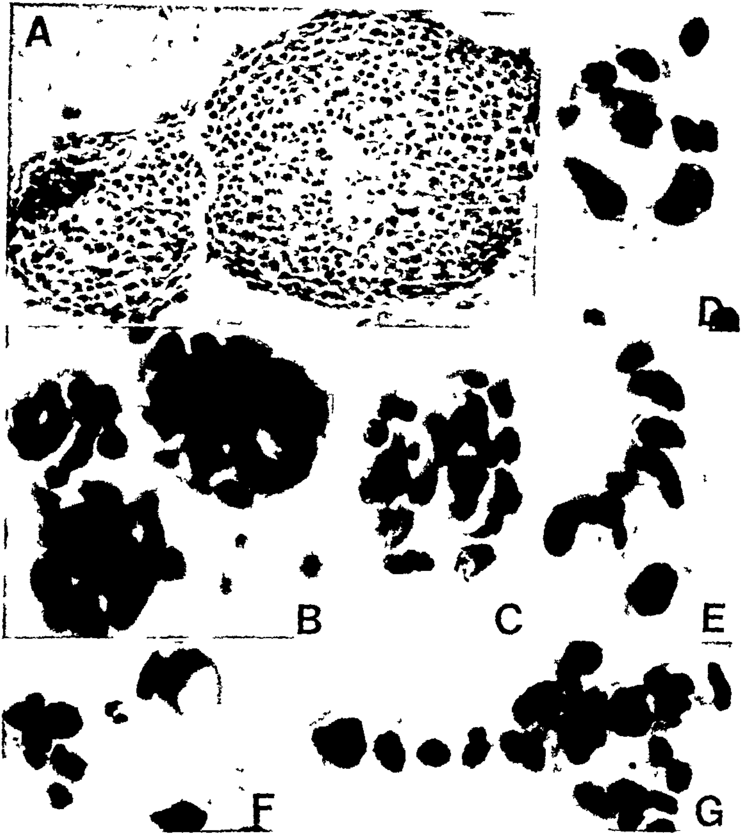


Figure 6

Metastatic epidermoid carcinoma pericardial effusion (A B) Exfoliative malignant epidermoid cells in smear Note the pseudoglandular arrangement of the tumor cells and their resemblance to *metastatic* adenocarcinoma (Papanicolaou stain × 570) (C) Exfoliated malignant epidermoid cells in cell block The tumor cells are arranged in a cohesive sheet The epidermoid nature of the tumor cells is more apparent in the cell block than in the smear (hematoxylin and eosin × 570)



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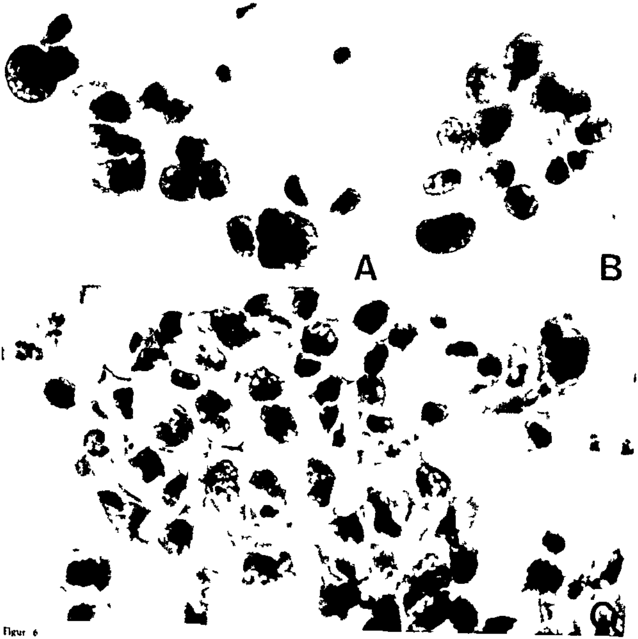

Figure 6
Metastatic epidermoid carcinoma in pericardial effusion (A B) Exfoliated malignant epidermoid cells in smear Note the pseudoglandular arrangement of the tumor cells and their resemblance to metastatic adenocarcinoma (Papanicolaou stain × 570) (C) Exfoliated malignant epidermoid cell in cell block The tumor cells are arranged in a cohesive sheet The epidermoid nature of the tumor cells is more apparent in the cell block than in the smear (hematoxylin and eosin × 570)

Table VI Clinical Data and Histologic Type of Primary Nonepithelial Tumors Metastatic to the Pericardium

	Age (years)	Sex	Total number of specimens	Number of positive specimens	Interval between 1st tissue diagnosis and follow up positive pericardial cytology (months)	Outcome
1 Mesothelioma	60	M	1	1	25	Died 3 mos
2 Mesothelioma	77	M	1	1	5	Died 20 mos
3 Mesothelioma	63	M	1	1	15	Alive 4 mos
4 Mesothelioma	62	M	2	1	27	Died 16 days
5 Malignant melanoma	62	M	2	2	6	Died 2 days
6 Malignant melanoma	40	M	1	1	23	Died 6 mos
7 Osteogenic sarcoma	20	M	2	2	34	Died 4 mos
8 Osteogenic sarcoma	13	M	2	2	12	Died 1 mo
9 Rhabdomyosarcoma	61	M	2	1	1	Died 20 days
10 Rhabdomyosarcoma	11	M	1	1	50	Died 2 mos
11 Hodgkin s disease	70	M	5	2	168	Alive 22 days
12 Leukemia	51	F	2	1	7	Died 6 mos
13 Lymphoma	61	F	1	1	15	Died 2 mos
14 Wilms s tumor	8	M	1	1	3	Died same day

Nonepithelial Neoplasms

There were 18 specimens from 14 patients (Table III) Clinical data and histologic types of the nonepithelial tumors are listed in Table VI Twelve patients were men and two were women

All four cases of mesothelioma were pleural in origin Malignant mesothelial cells were rather large and occurred singly and in clusters or morulae Nuclei were eccentric hyperchromatic with coarse clumps of chromatin and prominent nucleoli and possessed rather prominent nuclear membranes Although the nuclei appeared malignant they were relatively uniform in size and shape The cytoplasm was moderate to abundant deeply acidophilic or basophilic and affected the color of the nucleus resulting in a low nuclear-cytoplasmic staining intensity The cytoplasmic membrane was rather indistinct (Figure 7)

Tumor cells of malignant melanoma were large round or oval and mostly single with only a few loosely arranged groups There was a marked variation in size of malignant melanocytes Nuclei were round or oval eccentric and hyperchromatic with coarse clumps of chromatin and prominent nucleoli They were single or multiple and had a rather regular nuclear contour Some of the nuclei contained intranuclear cytoplasmic invaginations Their cytoplasm was abundant and darkly stained with a well-defined cytoplasmic membrane (Figure 8)

The other nonepithelial tumors were too few to permit any generalizations about the cytology of the individual types In general the cytomorphology of nonepithelial tumors in pericardial fluid was similar to that in other body cavity fluids (Figure 9)

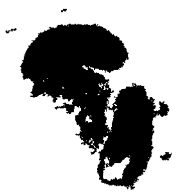

Figure 7
A solitary neoplastic cell of epithelioid mesothelioma metastatic to the pericardium (Papanicolaou stain × 570)

Effusions without Cancer Cells

There were 53 cytologically negative pericardial

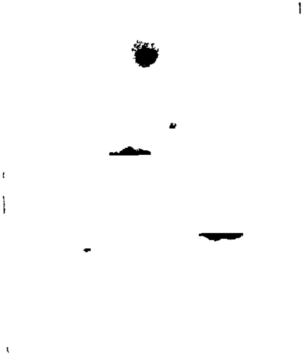

Figure 8
A large binucleated tumor cell of metastatic malignant melanoma in pericardial effusion (Papanicolaou stain × 570)

Figure 9
Exfoliated cells of metastatic rhabdomyosarcoma in pericardial effusion (Papanicolaou stain × 570)

specimens collected from 48 patients All 48 patients had been treated for malignant neoplasms In 2 of the 48 patients with negative specimens pericardial biopsy revealed metastatic neoplasms On review of the cytologic specimens no malignant tumor cells were identified The specimens obtained from 23 patients showed some mesothelial atypia Twelve patients had severe mesothelial atypia with occasional papillary formation cellular and nuclear enlargement nuclear hyperchromasia with coarse clumps of chromatin sometimes prominent nucleoli and mitotic figures (Figure 10) The cells had darkly stained cytoplasm that was lacy at the periphery and they were always identifiable as mesothelial in origin A few of the patients with prior treatment either radiation or chemotherapy showed an unusually marked degree of mesothelial atypia with cellular and nuclear enlargement abundant cytoplasm and markedly irregular cytoplasmic membrane (Figures 10A and 10B) The background usually contained inflammatory cells particularly lymphocytes Only 5 of 53 specimens had blood in the background

A most striking example of mesothelial atypia which was originally considered suspicious for a malignant tumor was seen in association with idiopathic hypertrophic subaortic stenosis (Figures 10C and 10D)

In 26 patients specimens were obtained during surgery and in many of them mesothelial cells showed the cytologic characteristics of specimens collected during operative procedures Flat sheets and groups of cells were common (Figure 10E) Cells were usually polygonal or sometimes columnar with eccentric oval or bean-shaped nuclei finely granular evenly distributed chromatin and one or multiple small nucleoli Nuclear membranes were generally smooth but occasionally showed marked irregularity Some of the clusters appeared crushed or compressed and contained hyperchromatic stripped nuclei Starch granules were commonly seen

Discussion

This study presents the cytomorphologic and clinicopathologic findings in 120 patients with pericardial effusion Malignant tumor cells were identified in 90 (57%) specimens from 72 (60%) patients One case originally reported as suspicious for malignant cells was interpreted on review as mesothelial atypia and subsequently proved to be due to idiopathic hypertrophic subaortic stenosis

The age of the patients with cytologically positive pericardial effusion ranged from 8 to 84 years with

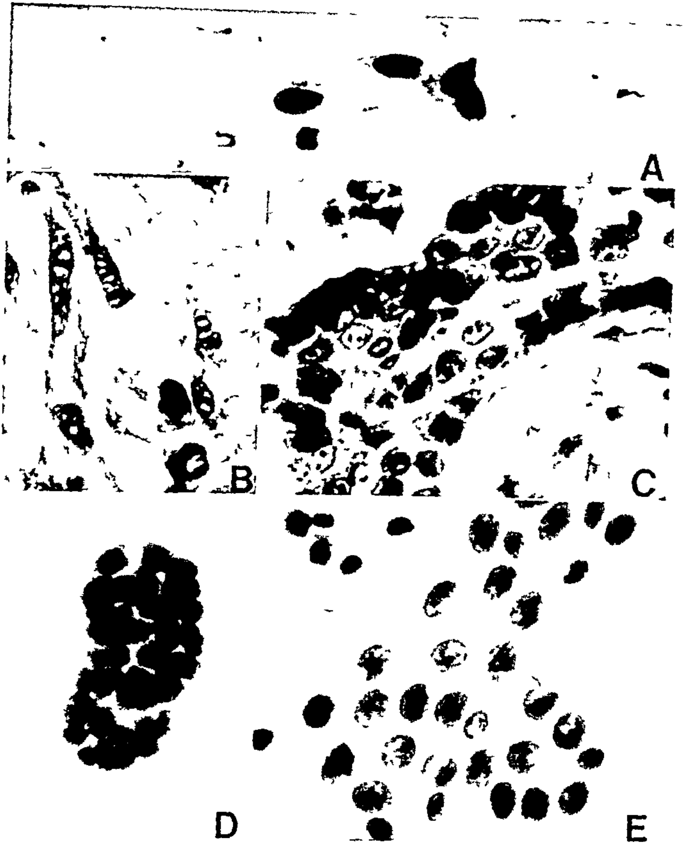

Figure 10

Mesothelial atypia. (A) Marked mesothelial atypia in pericardial fluid from a patient with history of radiation therapy. Note the branching cytoplasm membrane (Papanicolaou stain, × 570). (B) Marked mesothelial atypia in a pericardial biopsy specimen following chemotherapy and a short course of irradiation (hematoxylin and eosin, × 570). (C) Sheet of markedly atypical mesothelial cells in a pericardial biopsy. Note marked cytoplasmic cohesion, uniform size and minute nucleoli (hematoxylin and eosin, × 570). (D) Cell ball of atypical mesothelial cells in a patient with idiopathic pericardial effusion (Papanicolaou stain, × 570). (E) Flat sheet of mesothelial cells in smear prepared from pericardial effusion obtained during surgery. Majority of the cells are polygonal. Nuclei are rather uniform with a finely granular chromatin. Most of the nuclei contain small nucleoli (Papanicolaou stain, × 570).

most between 41 and 70 years and an average of 54 years There were 40 men and 32 women Eighty per cent of the tumors were epithelial and 20% were nonepithelial The most common epithelial tumors were pulmonary (56%) and mammary (39%) carcinomas *This can be explained partly by their proxim*ity to the pericardium and the possibility of spread by direct extension Histologically 77% of the epithelial tumors were adenocarcinomas and 12% were epidermoid carcinomas Pleural mesothelioma was the most common nonepithelial tumor

In most instances there was an interval of several months between the initial diagnosis of the primary neoplasm and the positive pericardial cytologic findings Patients with mammary carcinoma had the longest interval with an average of 48 months

The presence of malignant pericardial effusion in patients with known cancer especially in cases without frank pericardial tamponade should not be regarded as a terminal phase of the disease [14] According to our study this is especially true of patients with metastatic mammary carcinoma to the pericardium Although the majority of patients died within ten months 15 patients survived one to two years

A positive pericardial effusion was the only cyto*logic evidence of neoplasm in 23 patients Thirty two* patients had cancer cells identified in cytologic specimens from other sites prior to the positive pericardial cytology The majority of those specimens were bronchopulmonary in origin or pleural effusions Seventeen patients had positive cytologic findings in specimens from other sites subsequent to the positive pericardial cytology Most of those specimens were pleural effusions

The cytomorphology of the adenocarcinomas in pericardial effusion was similar to their morphology in other body cavity fluids [3,4,11] In general nuclear pleomorphism and prominence of nucleoli were dominant features of pulmonary adenocarcinoma Tumor cells of mammary carcinoma were rather uniform and often formed cell balls A small percentage of mammary carcinomas had a striking resemblance to adenocarcinoma of the lung Indian file arrangement was a nonspecific finding and was noticed in small cell adenocarcinomas of breast and lung oat cell carcinoma and carcinoid tumor *Although mesothelioma* is rather uncommon it can sometimes cause difficulty in differentiating from the far more common adenocarcinomas A number of features are helpful In mesothelioma the tumor cells belong to one population showing transition from benign to malignant and they do not stain positively with mucicarmine In contrast effusions with adenocarcinoma show two populations of cells neoplastic and mesothelial with no transitional form and the neoplastic cells may stain positively with mucicarmine Epidermoid carcinomas were poorly differentiated and often difficult to distinguish from adenocarcinoma This was particularly true in smears malignant epidermoid tumor cells showed better-defined squamous features in cell blocks In oat cell carcinoma the tumor cells were small with occasional medium-sized mononucleated cells Nucleoli were present in many of the oat cells The cytomorphology of nonepithelial tumors in pericardial fluid was similar to that in other body cavity fluids [4]

Lymphoreticular tumors were fewer than expected in this series perhaps because pericardiocentesis was not as critical for management of patients as with some of the other tumors Eight of 120 patients had lymphoma or leukemia and only 3 of the 8 had cytologically positive specimens

It is particularly worth noting that reactive cells such as lymphocytes and histiocytes were relatively rare in cytologically positive specimens In contrast the background of the negative specimens usually showed inflammatory cells especially lymphocytes *Another major difference was the presence of a* bloody background in the majority of the cytologically positive specimens whereas it was present in only 9% of the negative pericardial effusions

Approximately 50% of the negative pericardial specimens showed mesothelial atypia In one half of these cases mesothelial atypia was severe enough to cause difficulty in cytologic interpretation but the cells always maintained their mesothelial appearance Mesothelial atypia was particularly troublesome in some of the patients with prior radiation or chemotherapy

Melamed and Cameron[10] described the cytologic characteristics of mesothelial cells in specimens obtained at laparotomy Similar morphologic changes were also observed in some of the specimens from pericardial effusions obtained at surgery and this would be considered at the time of cytologic interpretation

As mentioned above 48 of 120 patients with peri*cardial effusion* had cytologically negative specimens In only two patients did follow up biopsy show metastasis to the pericardium and there was no evidence of metastasis either histologically or clinically in the remaining 46 Pericardial effusion in patients with cancer does not necessarily signify metastasis to the heart and the diagnosis should be con firmed by cytologic or histologic means if metastatic neoplasm is suspected clinically On the other hand as indicated by Zipf and Johnson[15] and observed in our material a negative report does not necessarily exclude the possibility of metastasis to the pericardium

References

1 Bisel HF Wroblewski F La Due JS Incidence and clinical manifestations of cardiac metastasis JAMA 153 712 715 1953

2 Flannery EP Gregoratos G Corder MP Pericardial effusions in patients with malignant disease Arch Intern Med 135 976-977 1975

3 Grunze H The comparative diagnostic accuracy efficiency and specificity of cytologic techniques used in the diagnosis of malignant neoplasm in serous effusions of the pleural and pericardial cavities Acta Cytol 8 150-163 1964

4 Hajdu SI Hajdu EO Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors Philadelphia WB Saunders 1976

5 Johnson WD The cytological diagnosis of cancer in serous effusions Acta Cytol 10 161 172 1966

6 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Philadelphia JB Lippincott 1979

7 Krikorian JG Hancock EW Pericardiocentesis Am J Med 65 808-814 1978

8 Lopes-Cardozo P A critical evaluation of 3 000 cytologic analyses of pleural fluid ascitic fluid and pericardial fluid Acta Cytol 10 455-460 1966

9 Martini N Freiman AH Watson RC Hilaris BS Malignant pericardial effusion NY State J Med 76 719 721 1976

10 Melamed MR Cameron MJ Benign mesothelial cells showing peritoneal irrigation artefact in a specimen obtained at laparotomy Cytopathology Check Sample No C 24 Chicago American Society of Clinical Pathologists 1975

11 Murphy WM Ng ABP Determination of primary site by examination of cancer cells in body fluids J Clin Pathol 58 479-488 1972

12 Ramsey SJ Tweeddale DN Bryant LR Braunstein H Cytologic features of pericardial mesothelium Acta Cytol 14 283 290 1970

13 Scott RW Garvin CF Tumors of the heart and pericardium Am Heart J 17 431-436 1939

14 Theologides A Neoplastic cardiac tamponade Sem Oncol 5 181 192 1978

15 Zipf RE Johnston WW The role of cytology in the evaluation of pericardial effusions Chest 62 593 596 1972

Amebae Resembling Entamoeba gingivalis *in the Genital Tracts of IUD Users*

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A total of 2 252 vaginopancervical (Fast) smears were studied as a continuation of an ongoing program to further investigate the occurrence of amebae in the genital tracts of women using intrauterine devices (IUDs) Of the 947 IUD users 1% harbored trophic amebae No amebae however were found in any of the 1 164 smears from non IUD wearers In all instances the amebae were associated with Actinomyces *Both organisms disappeared promptly after removal of the IUD In preparations stained according to the Papanicolaou iron hematoxylin and Gomori Wheatley trichrome methods the amebae could be diagnosed as belonging to the genus* Entamoeba *These trophozoites failed to stain with fluorescein labeled anti* Entamoeba histolytica *serum Although on the basis of the presently available evidence these trophozoites cannot be assigned with certainty to any known species they appear to share many morphologic characteristics with* Entamoeba gingivalis

Observations of amebae in the female genital tract are relatively uncommon Fentanes de Torres et al [10] in surveying the world literature found records of 83 proven cases and added 40 cases of their own In each case the ameba was identified as *Entamoeba histolytica* on the basis of its structure the clinical nature of the genital infection and the concomitant presence of the organism in the intestine

Amebic trophozoites in the genital canal of a patient using an intrauterine device and residing in a geographic area of low endemicity for *E histolytica* were first reported in 1978 [17] *Actinomyces* colonies were also present Removal of the IUD promptly resulted in the clearing of both organisms The current study has been conducted to gather additional information on the prevalence of these infections

Materials and Methods

One thousand eighty-eight genital tract specimens from 947 women wearing intrauterine devices were examined Eight hundred eighty-eight of the specimens were from 813 females from the Baltimore

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Structure of Ameba Trophozoites

The trophozoites measured 15μm to 60μm in the routinely obtained gynecologic material wet fixed with 95% ethanol or spray fixative or in Bouin's solution (Figure 2) Variation in size was not related to type of fixation or stain employed

In the Papanicolaou stain the cytoplasm of the ameba was pale blue and finely granular Pseudopods when present were clear blunt and frequently multidirectional The vesicular nucleus 3μm to 4μm in diameter was eosinophilic The peripheral chromatin granules and endosome were clearly visible In all 13 cases the organisms contained a varied number of cytoplasmic inclusions 3μm to 4μm in diameter They were generally rounded or lobulated stained darkly with hematoxylin and appeared to be ingested remnants of degenerated leukocytes within food vacuoles (Figures 2C and 2D) No ingested red cells were identified in any of the amebae even when found in a bloody background (Figure 2F)

The cytoplasm was also pale blue in the Gomori Wheatley trichrome stain but it appeared gray in iron hematoxylin-stained organisms The blunt multidirectional pseudopods (Figure 2E) were sharply demarcated from the endoplasm in both of these stains The cytoplasmic inclusions and nuclear elements stained brick red with trichrome and black with iron hematoxylin The nucleus was vesicular consisting of a distinct membrane lined with chromatin granules and a relatively small compact endosome the identifying characteristics of Entamoeba [9,20] In some nuclei the peripheral granules were uniform in size and evenly spaced in others there was considerable variability in both features Typically the endosome was composed of several irregularly shaped granules and it was central or eccentric Occasionally a surrounding cloudy halo was visible A linin network in the form of fine radiating filaments was commonly observed (Figures 2A 2B 2E and 2F)

Table II *Clinical Data*

Case	Age	Residence	IUD usage		Pertinent medical history
			Type	Years	
1†	30	Baltimore MD	Lippes loop	7	Regional enteritis Rx Resection of terminal ileum prednisone therapy
2‡	32	Baltimore MD	INCON	11	Right tuboovarian abscess Rx Total hysterectomy bilateral salpingectomy and right oophorectomy
3‡	32	Baltimore MD	INCON Dalkon shield	5 7	Mild hypertension
4	31	Baltimore MD	INCON	14	Pelvic inflammatory disease Rx Total hysterectomy and bilateral salpingectomy and oophorectomy
5	34	Boston MA	Lippes loop	8	Nothing significant
6	40	Detroit MI	Lippes loop	7	H/O swimming in sewage-contaminated water in Louisiana episodes of diarrhea for one year
7	33	Baltimore MD	Dalkon shield	4	Treated with prednisone for a positive LE prep
8	46	Baltimore MD	Dalkon shield	4	Psychomotor epilepsy since childhood Rx Aldactazide Mysolin Dilantin Librium Phenobarbital Dysfunctional uterine bleeding Rx Total abdominal hysterectomy and bilateral salpingectomy and oophorectomy
9‡	47	Washington DC	Lippes loop	9	IUD string not seen by gynecologist [illegible] X ray showed an IUD still in place
10	36	Newark NJ	?	4	Nothing significant
11	30	Washington DC	?	6	Nothing significant
12	31	Kansas City MO	?	4	Nothing significant
13	25	Harpers Ferry WV	?	4	Inmate of Federal Ref[illegible]

Age at time of detection of amebae in genital tract

Frequently the organisms were numerous (more than 100 per preparation) and usually appeared on a background of marked inflammation with many neutrophils The amebae occurred in close association with *Actinomyces* (Figure 2C) and also scattered throughout the entire specimen No cystic forms were observed

Clinical Data

The ages of the women harboring amebae in their genital tracts varied from 25 to 47 averaging 35 years (Table II) Six patients resided in Baltimore Maryland two in Washington DC one in Detroit Michigan one in Boston Massachusetts one in Newark New Jersey one in Kansas City Missouri and one in Harpers Ferry West Virginia None had a history of traveling outside the country within the two years before amebae were detected in their genitalia One patient however had a history of swimming in sewage polluted water in Louisiana one year before the cytologic examination she has had occasional episodes of diarrhea since that time Repeated parasitologic stool examinations of this patient as well as in two other cases in this series were negative for amebae

Of the IUDs employed four were Lippes loops [13] three were Dalkon shields [7] and three were INCON devices[8] the exact type was not known in four cases The devices had been in place an average of seven and a half years (four to 14 years Table II)

In addition to the presence of an IUD significant clinical history included corticosteroid therapy in two patients one for Crohn s disease[17] and the other following a laboratory report of a positive L E test One patient was on anticonvulsive drugs for psychomotor epilepsy Two patients had a history of chronic pelvic inflammatory disease requiring surgical treatment There was a case of mild arterial hypertension (Table II)

Cytopathologic studies prior to IUD insertion were available for review in cases 2 and 3 (Figure 3) *Actinomyces* and amebae were not detected on four different occasions before insertion of the device Repeat cytologic examination following IUD removal was available for study in eight of these 13 women (cases 1 to 8) Neither of the organisms was present in 14 different samples which were taken after removal of the device

All 13 women had amebae and *Actinomyces* while the IUDs were still in place No amebae were present in four isolated specimens from three patients In one of these patients (case 3) a sample taken three weeks following insertion of the IUD contained neither *Actinomyces* nor amebae Both organisms were present 12 years later Two months after treatment with metronidazole (Flagyl) the amebae disappeared but *Actinomyces* remained In two other patients (cases 2 and 10) the sample taken five and three years respectively after IUD insertion contained only *Actinomyces* while samples taken 11 and four years respectively after insertion of the device had both organisms (Figure 3)

Pertinent Clinical Abstracts

Case 2 Age 32 Baltimore resident Fast smears in May 1965 during pregnancy and in December 1965 six weeks postpartum contained *Trichomonas vaginalis* An INCON device was inserted in February 1966 When the patient next returned in October 1970 complaining of lower abdominal pain the smear contained *Actinomyces* and *Neisseria gonorrhea* She received antibiotic therapy for pelvic inflammatory disease and was seen on several subsequent occasions for recurrent episodes Cytologic examination on July 6 1977 and on August 19 1977 (11 years after IUD insertion) revealed *Actinomyces Entamoeba Trichomonas* and *Candida* The IUD was removed on August 22 and a specimen the following day contained no *Actinomyces* or *Entamoeba* however *Trichomonas* and *Candida* remained By September 2 after metronidazole and nystatin therapy there was clearing of all infections Total hysterectomy bilateral salpingectomy and right oophorectomy were performed on November 25 1977 for chronic pelvic inflammatory disease

Case 3 Age 32 Baltimore resident An INCON device was inserted on August 11 1965 Fast smears on August 11 and September 2 1965 revealed no inflammation or pathogens The INCON device was removed and replaced by a Dalkon shield on January 26 1970 and a third device replacement was carried out on October 19 1972 for a new Dalkon shield A third Fast smear on May 10 1977 (12 years of IUD use) revealed marked inflammation and the presence of *Trichomonas Actinomyces* and *Entamoeba* After metronidazole therapy a cytologic specimen on July 3 revealed no *Trichomonas* or *Entamoeba* but marked inflammation and *Actinomyces* remained The IUD was removed on July 25 and the cytologic material taken on October 5 1977 revealed neither inflammation nor *Actinomyces*

F E H U Boston Massachusetts Dr Ringgold P H S Outpatient Clinic Detroit Michigan Dr J Salva F E H U Newark New Jersey and P M Wood R N F E H U Washington DC for clinical follow ups on patients included in the study F La *Corte for the photographic work and N Weaver D* Blackburn and B Franza for typing the manuscript

References

1 Bhaduri KP *Entamoeba histolytica in leukorrhea and salpingitis* Am J Obstet Gynecol 74 434 1957

2 Belding DL Textbook of Clinical Parasitology Second edition New York Appleton-Century-Crofts 1952 p 98

3 Brandt H Perez Tamayo R Pathology of human amebiasis *Hum Pathol 1 351 385 1970*

4 Carter B Jones CP Thomas WL Invasion of squamous cell carcinoma of the cervix uteri by *Entamoeba histolytica* Am J Obstet Gynecol 68 1607 1610 1954

5 Chandler AC Read CP Introduction to Parasitology Tenth *edition New York John Wiley & Sons 1961 pp 81-82*

6 Christ ML Haria J Cytologic changes associated with vaginal pessary use Acta Cytol 22 146-149 1978

7 Davis HJ The shield intrauterine device A superior modern contraception Am J Obstet Gynecol 106 455-456 1970

8 Davis HJ Lesinski J Mechanism of action of intrauterine contraceptives in women Obstet Gynecol 36 350 358 1970

9 *Faust EC Beaver PC Jung RC* Animal Agents and Vectors of Human Disease Fourth edition Philadelphia Lea & Febiger 1974 pp 70-71 77

10 Fentanes de Torres E Benitez Bribiesca L Cytologic detection of vaginal parasitosis Acta Cytol 17.252 257 1973

11 *Goodey T Wellings AW* Observations on *Entamoeba gingivalis* from the human mouth with a note on the trichomonad flagellate *Tetratrichomonas buccalis* n sp Parasitology 9 537 559 1917

12 Gupta PK Hollander DH Frost JK Actinomycetes in cervico vaginal smears An association with IUD usage Acta Cytol 20 295-297 1976

13 Lardner TJ Shack WJ Tam LY IUD Information Handbook Boston The Pathfinder Fund 1970

14 Lomax CW Harbert GM Jr Thorton N Jr Actinomycosis of *the female genital tract* Obstet Gynecol 48 341 346 1976

15 Luff RD Gupta PK Spence MR Frost JK Pelvic actinomycosis and the intrauterine contraceptive device Am J Clin Pathol 69 581 586 1978

16 MacCarthy J Actinomycosis of the female pelvic organs with *involvement of the endometrium J Pathol Bacteriol 69 175-178* 1955

17 McNeill RE deMoraes Ruehsen M Ameba trophozoites in cervical vaginal smear of a patient using an intrauterine device A case report Acta Cytol 22 91 92 1978

18 Munguia H *Franco E Valenzuela P Diagnosis of genital* amebiasis in women by the standard Papanicolaou technique Am J Obstet Gynecol 94 181 188 1966

19 O'Brien PK Abdominal and endometrial actinomycosis associated with an intrauterine device Can Med Assoc J 112 596-597 1975

20 Spencer FM Monroe LS The Color Atlas of Intestinal Parasites Sixth edition Springfield Illinois Charles C Thomas *1975 pp 27 38 52*

21 Weinstein BB Weed JC Amebic vaginitis Am J Obstet Gynecol 56 180-183 1948

22 Wenyon CM Protozoology A Manual for Medical Men Veterinarians and Zoologists New York William Wood & Co *1926 pp 184 225*

23 Westrom L Bengtsson LP Mardh P The risk of pelvic inflammatory disease in women using intrauterine contraceptive devices as compared to non-users Lancet 2 221 224 1976

Cerebrospinal Fluid Cytology in Five Patients with Cerebral Cysticercosis

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Cerebral cysticercosis from Taenia solium *infection is a common disease in Central American countries but an infrequent one in the United States We report distinctive cellular findings in cerebrospinal fluid (CSF) and attendant clinical features in five patients from Central America The patients histories showed variable previous parasitic infestations their neurologic symptoms and signs usually were nonfocal Routine examination of CSF of the five patients revealed elevated leukocyte counts but eosinophilia was found in only two Three of the CSFs showed marked pleocytosis high variability and atypia indicating possible lymphoma of the central nervous system No hooklets or other structure associated with parasitic invasion were identified The clinical findings together with the cellular pleomorphism in CSF generally suggested an inflammatory lesion rather than lymphoma In cerebral cysticercosis confirmation of diagnosis requires proof of the etiologic agent*

Cysticercosis the human infection by larvae of the pork tapeworm *Taenia solium* is a common disease in Latin America Asia Africa Eastern Europe and parts of Western Europe [5] In the United States tapeworm infestation occurs infrequently However increasing immigration and world travel are bringing individuals who harbor the disease into geographic areas where it is infrequent It is therefore helpful to bring its manifestations to the attention of the clinician

We report on the cytology of the cerebrospinal fluid (CSF) of five patients with suspected cysticercosis

Case Reports

Patient 1

A 28 year-old woman who lived in El Salvador most of her life had complained of frequent headaches and blurred vision since she was six years old Her condition at that time was diagnosed as hydrocephalus and a right parietal shunt was performed Her condition improved especially after steroid therapy She visited the United States for periodic evaluation and treatment On a recent visit to San Francisco the vision in her right eye had deteriorated further and she complained of increased headaches Her relatives noted her emotional instability and failing memory Her only significant physical finding was chronic papilledema

Laboratory findings are shown in Table I Workup including CT scan and pneumoencephalography revealed multiple cysts in the area of the quadrigeminal cistern CSF complement fixation titer for cysticercosis was 1 64 Cytologic examination was performed on 8 ml of clear slightly yellow CSF which had a specific gravity of 1 005 Most cells were lymphocytes there also were cells with large round nuclei prominent nucleoli scant cytoplasm (Figure

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able I CSF Findings in Five Patients with Cerebral Cysticercosis

atient						Concentrated smear (100 cells)				
CSF	RBC (cu mm)	WBC (cu mm)	Glucose (% mg)	Protein (% mg)	Polymorphonuclear cells	Eosinophils	Lymphocytes	Monocytes histiocytes	Undetermined cells
Clear	0	68	57	30	0	1	87	7	5
Hazy	980	40	52	142	62	0	33	5	0
						Initial CSF 41			
Light yellow	2	375	16	780	1	6	93	0	0
Clear	3	94	51	54	0	0	100†	(100)†	0
Clear	Few	250	21	260	0	0	97	3	0

Not done in our laboratory
Lymphocytes, monocytes and histiocytes combined

Figure 1
Patient 1. Heterogeneous cell population with lymphocytes, atypical convoluted lymphocytes and several cells with scant cytoplasm and prominent nucleoli (original magnification × 250).

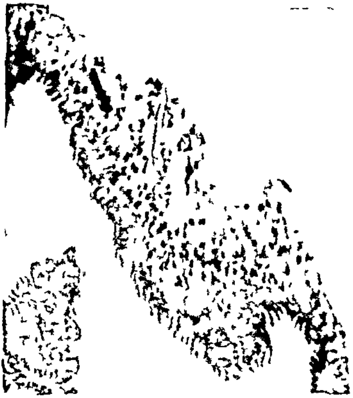

Figure 2
Patient 1. Cyst wall showing three layers: (1) microvillous lining, (2) cellular zone and (3) loose fibrous connective tissue (original magnification × 63).

1) and occasional histiocytes, but there were no polymorphonuclear leukocytes (PMNs) or eosinophils (Table II). No hooklets were seen. The patient underwent craniotomy, and the cysts were removed. Pathologic examination showed the cyst walls to be the typical three-layered configuration of cysticerci (Figure 2). Follow-up stool examinations for ova and parasites revealed rare eggs of *Trichuris trichiura* and *Taenia* sp. The patient returned to El Salvador for follow-up.

Patient 2

A 37-year-old man working for United Airlines had emigrated from Nicaragua to the United States at age 21. At age 7 he reportedly had tapeworm as well as *Ascaris lumbricoides* infection. He had a history of episodic headaches, fever, chills and general malaise associated with a stiff neck. CSF evaluation at another hospital had yielded 41% eosinophils in the differential leukocyte count. Workup revealed an avascular mass in the right temporal lobe. CSF com

Table II *CSF Cytology in Five Patients with Cerebral Cysticercosis*

Patient	Lymphocytes	Plasma cell	Histiocytes	Atypical mononuclear cells	Undetermined cells	Polymorphonuclear cells	Eosinophils	Total no cells
1	300	0	4	25	0	0	0	3 9
2	56	0	3	3	10	31	0	103
3	300	22	21	13	0	6	0	362
4								
5	64	12	28	50	10	0	0	164

Clumps with predominant lymphocytes histiocytes atypical lymphocytes and occasional polymorphonuclear cells

plement fixation titer for cysticercosis was 1 4 096 The patient s symptoms were controlled with steroid therapy On the day of admission he had three right sided focal seizures There were no remarkable localized neurologic findings

Laboratory evaluation of CSF obtained by lumbar puncture is shown in Table I Cytologic evaluation was performed on 2 ml of slightly yellow CSF Most cells were lymphocytes a few atypical mononuclear cells and PMNs were seen (Table II) The patient underwent craniotomy and the cysts were removed Pathologic examination showed the three layered cyst wall of cysticercosis The patient shows persistent neurologic deficit

Patient 3

A 36 year-old Mexican male recently immigrated to the United States He was referred to our institution after being evaluated elsewhere for a one month history of persistent vomiting accompanied by chills Physical findings included slight ataxia In the hospital he had a grand mal seizure Stool was negative for ova and parasites Fungal and tuberculosis cultures of CSF were negative The CSF complement fixation titer for cysticercosis was 1 25 600

CSF laboratory findings are shown in Table I Cytologic examination was performed on 5 ml of slightly yellow CSF Most cells were lymphocytes scattered histiocytes plasma cells and atypical mononuclear cells were also present (Table II) Arteriography and pneumoencephalography revealed no cranial defects The patient s condition improved with steroid therapy and he was referred for continuing follow up

Patient 4

A 31 year-old Mexican male had a four to five month history of frontal headaches dizziness diplopia blurred vision bilateral weakness nausea and vomiting The only significant physical finding was chronic papilledema CSF VDRL *Cryptococcus Histoplasma Toxoplasma* and coccidiosis antibodies were negative The CSF cysticercosis complement fixation titer was negative Stool examination for ova and parasites revealed *Entamoeba histolytica* and *Taenia* ova

Laboratory evaluation of CSF is shown in Table I CSF cytology showed few scattered clumps of cells predominantly lymphocytes and histiocytes with occasional atypical lymphocytes and PMNs (Table II and Figure 3) A CT scan showed diffuse cerebritis and arachnoiditis with a right parietal lesion having

Figure 3
Patient 4 Edge of cell clump showing cellular pleomorphism with lymphocytes and atypical mononuclear cells Compare with Figure 5 (original magnification × 250)

a ringlike configuration. The patient underwent brain biopsy; subsequent pathologic examination showed meningoencephalitis and cerebral cysticercosis (Figures 4 and 5). The patient had previously been treated with an antiparasitic drug. He returned to Mexico.

Patient 5

A 44-year-old Mexican woman immigrated to the United States at age 26. She had a long history of mild gait disturbances and seizures. Her relatives had noted a change in her affect, which included dramatic mood alteration, depression and apparently hysterical behavior. On neurologic examination the patient had a flat affect with disorientation to time and place. The cranial nerves and the sensory examination were within normal limits. Reflexes were normal, but there was a right-sided snout and grasp reflex. There was increased muscle tone.

CSF findings are shown in Table I. An EMI scan revealed a right parietal calcified lesion. CSF cytology was performed on multiple occasions; the findings are shown in Table II. Many cells were atypical (Figure 6). CSF cultures for fungi, tuberculosis and bacteria were negative. CSF cysticercosis titer was 1:512. The patient was discharged for psychiatric follow-up and receives anticonvulsant therapy.

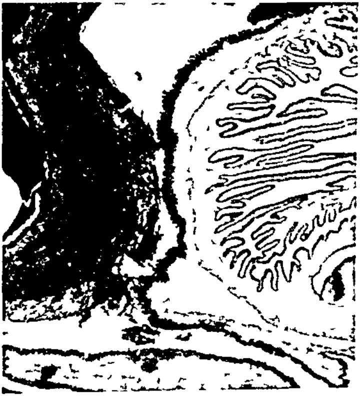

Figure 4
Patient 4. Low-power view of brain biopsy specimen. The parasitic cyst is on the right, and the inflammatory response is in the vertical border of tissue at the left. A higher-power view of the inflammation is seen in Figure 5 (original magnification × 10).

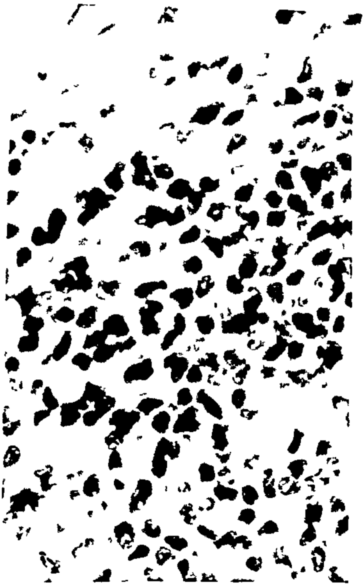

Figure 5
Patient 4. A higher-power view of the inflammatory infiltrate seen in Figure 4. Note the pleomorphism and atypism of the mononuclear cells (original magnification × 250).

Discussion

Humans acquire *T. solium* infection by ingestion of undercooked pork, which often contains the encysted larvae (cysticerci) of *T. solium*. The larva develops into the adult tapeworm in the human gastrointestinal tract. Eggs or egg-bearing segments are passed with the feces and may be eaten by pigs. In the pig gut the eggs develop into motile larvae, which migrate through the pig's bloodstream to muscle and soft tissue. In humans who ingest the eggs from hu man fecal contamination the eggs may hatch and release motile larvae that penetrate the bowel and spread to other tissues including skin muscle and brain[6] just as occurs in the pig The motile larvae then enlarge and develop into a fluid filled bladder containing a future adult scolex the cysticercus

Each of our patients had a cerebral infection by such cysticerci of *T solium* Although the disease is rare in the United States the geographic origin of these five patients suggests a certain population at risk for cysticercosis They all lived in Central America a region endemic for *T solium* Two patients had had multiple infestations of other parasites Our clinical findings were variable and included psychologic disturbances meningeal signs hydrocephalus and seizures Three distinct syndromes have been reported[2,3] (1) localized cystic lesions with localized signs seizures and obstructive hydrocephalitis (2) widespread infection and increased intracranial pressure and (3) proliferative inflammatory reaction and basal meningitis with fever and signs of meningeal irritation There is extensive literature describing the presence of CSF pleocytosis in connection with cysticercosis[1,2,4,5,7,9] Our five patients exhibited a wide range of cytologic features and shared certain clinical features

CSF eosinophilia has been emphasized as suggestive of parasitic involvement of the central nervous system (CNS)[3,4] Only two of our patients showed significant eosinophilia and one of the two showed no eosinophils on repeat examination Eosinophilia however is not a constant finding in cysticercosis The two patients had elevated leukocyte counts and the significant cells were predominantly mononuclear in morphology The CSF protein content was usually elevated

Cytologic examination of the CSF encompasses a wide range of cellular morphology As expected no hooklets suggestive of *T solium* were found Most cells from each patient were lymphocytes Few polymorphonuclear cells were seen Occasionally we identified histiocytes and plasma cells The significant cells appeared to be atypical mononuclear lymphoid cells (Figures 1 and 6) In patients 1 3 and 5 the presence of these cells raised the possibility of a lymphoma of the central nervous system In these patients cell variability and atypia were extreme but none showed clinical evidence of lymphoma The distinctive features were atypical reactive lymphoid cells in conjunction with a *mixed* cellular population The interpretation of these findings was that of a benign reactive process in the central nervous system

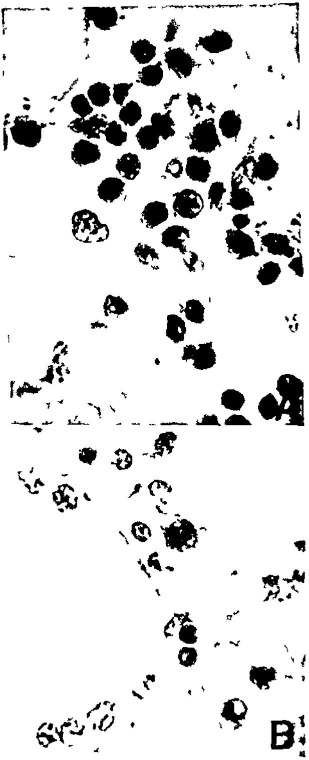

Figure 6
(A and B) Patient 5 Atypical mononuclear cells showing atypical features including coarse chromatin clumping and convolution Several cells have prominent nucleoli (original magnification × 250)

Ultimate diagnosis required surgical biopsy and pathologic evaluation of biopsied material as well as complement fixation tests on CSF for *T solium* Radiographic evaluation including CT scan provided additional evidence of lesions in the CNS Helpful criteria suggested for diagnosis of CNS cysticercosis[2,3] are (1) patient migrating from an endemic area (2) eosinophilia of blood (3) eosinophilia of CSF (4) CSF lymphocytosis (5) elevated CSF protein level (6) intestinal infection (7) positive complement fixation test and (8) calcifications on roentgenogram The Center for Disease Control considers the indirect hemagglutination (IHA) and the fluorescent antibody tests more reliable means of serologic diagnosis than the positive complement fixation test

Our experience with these five cases has shown that cerebral inflammatory lesions due to cerebral cysticercosis yielded a CSF cytologic pattern of polymorphism in which some of the more atypical manifestations raised a differential consideration of possible CNS lymphoma The cytologic heterogeneity in all five patients favored a benign diagnosis and eosinophilia was not a prominent feature In the differential diagnosis cerebral cysticercosis must be considered for patients with appropriate clinical symptoms and when dealing with populations at risk However cysticercosis is only a differential possibility until definitive evidence of the presence of the etiologic agent is obtained

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References

1 Danziger J Bloch S Tapeworm cyst infestations of the brain Clin Radiol 26 141 148 1975

2 Escobar A Cerebral cysticercosis N Engl J Med 298 403-404 1978

3 Hoffman SF Guthrie TH Cerebral cysticercosis South Med J 68 105 108 1975

4 Lombardo L Mateos JH Cerebral cysticercosis in Mexico *Neurol 11 824-828* 1961

5 Marquez Monter H Cysticercosis *In* Pathology of Protozoal and Helminthic Diseases Edited by RA Marcial Rojas Baltimore Williams & Wilkins 1971 pp 592-617

6 Olive JI Angulo Rivero P Cysticercosis of the nervous system I Introduction and general aspects J Neurosurg 19 632-634 1962

7 Schnur JA Richardson EP Jr Progressive dementia and seizure disorder in a 62 year-old native of the Cape Verde Islands N Engl J Med 297 773-780 1977

8 Spriggs AI Boddington MM The Cytology of Effusions—*Pleural Pericardial and Peritoneal—and of* Cerebrospinal Fluid Second Edition New York Grune & Stratton 1968 pp 43-69

9 White JC Sweet WH Richardson EP Cysticercosis cerebri N Engl J Med 256 479-486 1957

Configuration of Surfaces of Human Cancer Cells Obtained by Fine Needle Aspiration Biopsy

A Comparative Light Microscopic and Scanning Electron Microscopic Study

Wenancjusz Domagala M D M I A C
Leopold G Koss M D F I A C

*The surface configuration of human cancer cells
moved from their natural setting by fine nee
aspiration biopsies was studied by scanning elect
microscopy Cells from several carcinomas of
breast malignant melanomas oat cell carcinon
and a malignant lymphoma were the subject of t
study Only viable cells identified by light micr
copy prior to scanning were selected for study C
trary to prior observations on cancer cells in ef
sions the surface configuration of human cancer c
obtained directly from tissues is variable and may
pend significantly on the histologic patterns*

Since the application of scanning electron mic
scopic techniques (SEM) to human exfoliated cells
Friedlander in 1969 [8] numerous studies of surface
trastructure mainly pertaining to cells in effusion
[5 7 9 11] and in the urine [4] have been published Th
studies suggested that in fluids differences in surf
configuration may be observed between cancer c
and various types of benign cells and raised the h
of a possible applicability of SEM to diagnosis I
viously developed techniques[3] used in the studie
surfaces of cells in fluids were now applied to
study of human cancer cells freshly removed fr
their natural setting by fine needle aspiration bi
sies

Materials and Methods

Aspiration biopsies of 15 primary breast carcinon
5 malignant melanomas (4 primary and 1 metasta
5 oat cell carcinomas and 1 malignant lymph
(large noncleaved cell type) were performed w
fine needles less than 1 mm in diameter Histol
cally all breast tumors were infiltrating duct ca
nomas in two of them gland formation was
served Four malignant melanomas were epith
cell type and one was spindle cell type [12]

The techniques developed in our laboratories
previously published[3] are based on light microsc
(LM) identification of cells prior to SEM study B
ly the aspirated cells were immediately suspende
a commercially available tissue culture med
(RPMI medium 1640 Grand Island Biological C
pany Grand Island New York) washed twic
RPMI by centrifugation for five minutes at 200 g
allowed to sediment onto a small area of a speci
prepared microcoverslip for 20 minutes at room t
perature The coverslips were fixed in 2% glutara
hyde in 0 1M cacodylate buffer (pH 7 4) for
hours washed in the same buffer and stained wi
modified Giemsa method The cells were identi

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References

1 Danziger J Bloch S Tapeworm cyst infestations of the brain Clin Radiol 26 141 148 1975

2 Escobar A Cerebral cysticercosis N Engl J Med 298 403-404 1978

3 Hoffman SF Guthrie TH Cerebral cysticercosis South Med J 68 105-108 1975

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6 *Olive JI Angulo Rivero P Cysticercosis of the nervous system* I Introduction and general aspects J Neurosurg 19 632-634 1962

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9 White JC Sweet WH Richardson EP Cysticercosis cerebri N Engl J Med 256 479-496 1957

Configuration of Surfaces of Human Cancer Cells Obtained by Fine Needle Aspiration Biopsy

A Comparative Light Microscopic and Scanning Electron Microscopic Study

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Leopold G Koss M D F I A C

The surface configuration of human cancer cells removed from their natural setting by fine needle aspiration biopsies was studied by scanning electron microscopy Cells from several carcinomas of the breast malignant melanomas oat cell carcinomas and a malignant lymphoma were the subject of this study Only viable cells identified by light microscopy prior to scanning were selected for study Contrary to prior observations on cancer cells in effusions the surface configuration of human cancer cells obtained directly from tissues is variable and may depend significantly on the histologic patterns

Since the application of scanning electron microscopic techniques (SEM) to human exfoliated cells by Friedlander in 1969 [6] numerous studies of surface ultrastructure mainly pertaining to cells in effusions[1,2,3,7,9,11] and in the urine [4] have been published These studies suggested that in fluids differences in surface configuration may be observed between cancer cells and various types of benign cells and raised the hope of a possible applicability of SEM to diagnosis Previously developed techniques[3] used in the studies of surfaces of cells in fluids were now applied to the study of human cancer cells freshly removed from their natural setting by fine needle aspiration biopsies

Materials and Methods

Aspiration biopsies of 15 primary breast carcinomas 5 malignant melanomas (4 primary and 1 metastatic) 5 oat cell carcinomas and 1 malignant lymphoma (large noncleaved cell type) were performed with fine needles less than 1 mm in diameter Histologically all breast tumors were infiltrating duct carcinomas in two of them gland formation was observed Four malignant melanomas were epithelial cell type and one was spindle cell type [12]

The techniques developed in our laboratories and previously published[3] are based on light microscopic (LM) identification of cells prior to SEM study Briefly the aspirated cells were immediately suspended in a commercially available tissue culture medium (RPMI medium 1640 Grand Island Biological Company Grand Island New York) washed twice in RPMI by centrifugation for five minutes at 200 g and allowed to sediment onto a small area of a specially prepared microcoverslip for 20 minutes at room temperature The coverslips were fixed in 2% glutaraldehyde in 0 1M cacodylate buffer (pH 7 4) for 12 hours washed in the same buffer and stained with a modified Giemsa method The cells were identified

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located and photographed under low and high power with a Zeiss automatic photoscope It must be stressed here that during this part of the procedure the cells were kept wet

After photography the cells on the microcoverslips were fixed in 1% OsO_4 in 0 1M cacodylate buffer (pH 7 4) and dehydrated in a series of acetones Following dehydration the cells were critical point dried in liquid CO_2 in a Polaron E3000 apparatus coated with 200 Å of gold palladium in an E5000 Polaron sputter coater and examined with a JEOL JSM 35 SEM at an accelerating voltage of 25 kv using a 200µm objective aperture and 120 mÅ beam current

Viability of Aspirated Cells

The use of aspiration biopsy for the evaluation of surface configuration of cells raises a question about the degree to which the aspiration procedure affects the viability of the examined cells The nuclei of glutaraldehyde fixed viable cells stained with the modified Giemsa method stained light blue the nuclei of necrotic cells stained deep purple Under the SEM the necrotic cells displayed surface defects of variable size and breaks in the cell membrane Approximately 80% of the cells obtained by fine needle aspiration biopsy appeared to be viable and only such cells were chosen for this study

Results

The aspirated material suspended in RPMI consisted of free cells and aggregates of cells The ratio of free cells to cell clusters varied from case to case The comparative fields in LM and SEM may be observed on the pairs of corresponding photographs made under low magnification as shown in Figures 1 to 5

Carcinoma of the Breast

The aspirates contained large clusters of cancer cells and single cancer cells The general configuration of cell clusters was usually irregular (Figure 1A) or approximately spherical (Figure 1D)

There were marked differences in shape and size of cells forming outer layers of the clusters The surface cells in some clusters were rather flat and tightly fitted while in other clusters the cells were clearly demarcated by invaginations of variable depth (Figure 1D) The outer surfaces of cells in clusters were covered by irregular microvilli (MV) which varied greatly in configuration size length and density (Figures 1B C E and F) On some cells the MV were numerous and densely packed (Figure 1B) Surface areas devoid of microvilli were also observed (Figures 1C and E)

Approximately 900 single cancer cells were scanned They were of greatly variable sizes and shapes but were mostly spherical The surface configuration of these cells was variable (Figures 2A and B) Long irregular surface MV short MV or cells devoid of MV were observed Of signal interest was the appearance of cancer cells with long MV confined to sharply demarcated areas of cancer cell surfaces (Figure 2C) It is likely that such MV-covered surface areas corresponded to the part of the cells facing the lumen of glandular structures in tissue cells of this type constituted more than 60% of all cancer cells in the gland forming breast cancer In such tumors approximately 10% of the remaining cells had a few sparse short MV (Figure 2D) The remaining cells had irregular surfaces devoid of MV

In breast cancers without obvious gland formation the majority of cancer cells displayed only a few MV scattered over the surface or an irregular surface without any MV (Figure 2F) There were only about 10% of cancer cells with long MV distributed over a limited area as described above

The mode of attachment of breast cancer cells to glass was by cytoplasmic filopodia or spikes of variable length (Figure 2E)

Malignant Lymphoma (Large Noncleaved Cell Type)

The material contained predominantly single cells and a few small clusters The cancer cells of which about 700 were scanned were predominantly spherical (Figures 3A and B) Approximately 70% of the cells were covered with irregular MV (Figure 3C) 20% with blebs of various sizes and shapes (Figures 3E to G) Some cells (5% to 10%) were covered with surface ridges Generally the malignant cells were attached to the glass by short cytoplasmic projections of slightly tapered form and uneven diameter (Figure 3D) Sometimes these cytoplasmic projections extended for considerable distances

Malignant Melanoma

Epithelial Cell Type Approximately 800 malignant cells were scanned The cells were either single or in small clusters The cancer cells varied greatly in size and shape within the same sample but many were spherical (Figure 4A) In two of the cases approximately 70% of the melanoma cells were covered with numerous long irregular MV and 25% with blebs of various sizes and shapes (Figure 4A) 5% had smooth

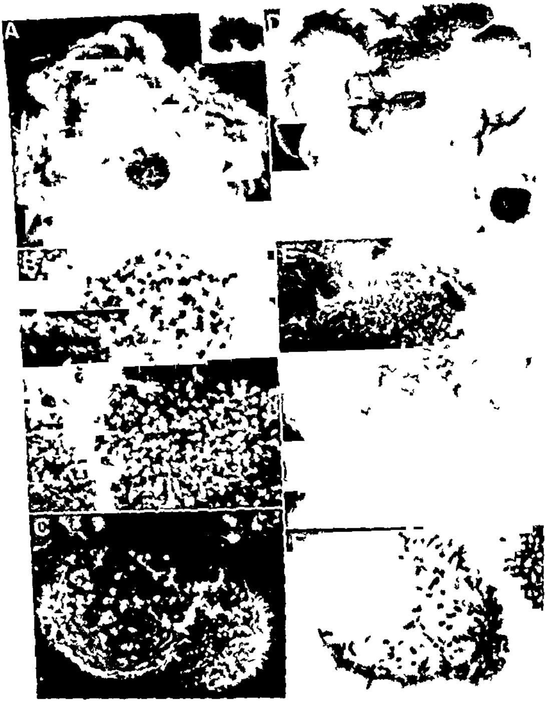

Figure 1
Infiltrating duct carcinomas of the breast (A) Cluster of cancer cells Note variation in size and shape of cells (× 800) Inset the same cluster in LM (× [illegible]00) *(B) Details of upper rectangle from Figure 1A* Two cancer cells covered by numerous irregular MV *(× 7 500) (C) Details of lower rectangle from Figure 1A* Note large areas devoid of MV (× 5 000) (D) Cluster of cancer cells *from another carcinoma of the breast* Note great variation in size and shape of cells (× 1 500) Inset the same cluster in LM (× 710) (E) Details of the rectangle from Figure 1D Note short MV and an area with no MV (arrows) *(× 5 700) (F) Details of the cell identified by triangle in Figure 1D* Note sparse MV of different length (× 9 000)

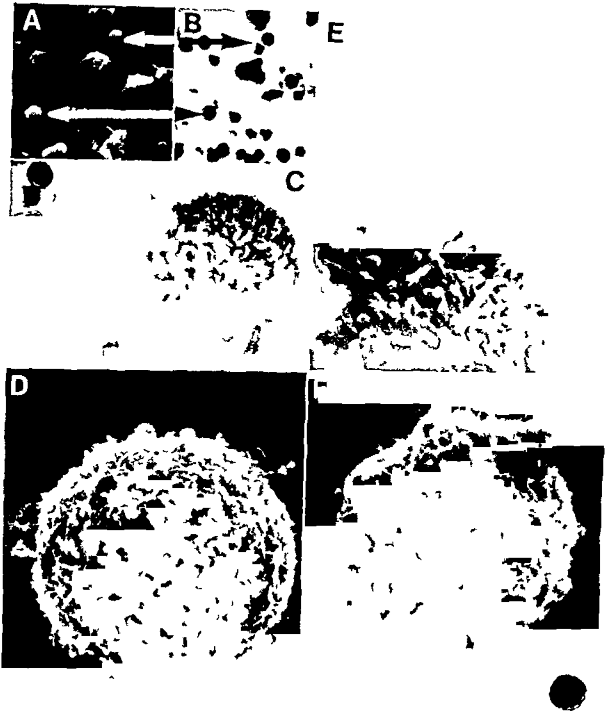

Figure 2

Infiltrating duct carcinomas of the breast (A B) Infiltrating duct carcinoma with gland formation Comparable SEM and LM locating pictures for Figures 2C and 2D (A × 420 B × 270) (C) Cell from the upper portion of Figures 2A and 2B (arrows) Numerous MV on the surface of cancer cell Note that the MV are present only on the upper portion of the cell The lower part of the cell surface is smooth (× 5 000) Inset LM of the same cell (× 540) (D) Cell from the lower portion of Figures 2A and 2B (arrows) Cancer cell with few MV scattered over the entire cell surface (× 6 800) (E) Infiltrating duct carcinoma with gland formation Thin cytoplasmic projections of variable length attach the cell to the glass (× 14 500) (F) Infiltrating duct carcinoma without gland formation Cancer cell with irregular surface configuration there are no MV (× 4 000) Inset LM of the same cell (× 570)

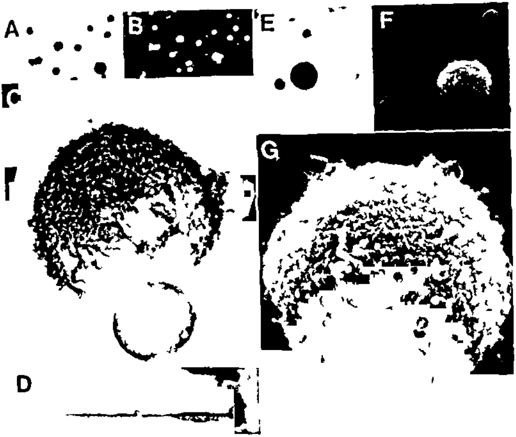

Figure 3
Malignant lymphoma (large noncleaved cell type) (A B) Low-power view of the same field in LM and SEM The cell shown in Figure 3C is in the lower right hand corner of Figures 3A and 3B (A × 190 B × 170) (C) Malignant cell covered with irregular MV The cell is attached to glass by long cytoplasmic projections Note the lymphocyte with changes suggestive of necrosis (bottom) (× 3 700) (D) High-power view of long cytoplasmic process marked by an arrow in Figure 3C attaching the cell to the glass (× 11 000) (E F) Low-power view of the same field in LM and SEM (A × 330 B × 660) (G) Malignant cell shown in E and F covered with numerous spherical blebs of various sizes and shapes (× 3 200)

surfaces In the remaining two cases approximately 60% of the cells were covered with blebs 20% to 30% had numerous MV and 10% had smooth surfaces In one case approximately 10% of the melanoma cells had a mixed surface configuration composed of blebs and short MV

Spindle Cell Type Approximately 200 malignant cells were scanned The elongated cells were either single or formed small clusters (Figure 4B) Approximately 60% of the cells had microvilli 30% showed blebs of various sizes and shapes and about 10% had smooth surfaces Malignant melanoma cells regardless of configuration were attached to the glass by thin long or short cytoplasmic projections Quite often the tip of the projection spread on glass forming lamellar cytoplasm

Oat Cell Carcinoma of the Lung

Cells of oat cell carcinoma were either single or in short chains (Figures 5A to C) or small clusters (Figure 5E) Approximately 500 cell images were scanned Cells in chains and clusters were tightly packed with cytoplasmic molding (Figures 5A and E). The cancer cells had generally smooth (Figure 5E) sometimes slightly wrinkled surfaces (Figure 5A) A few cells showed sparse, short, stubby MV but generally MV were not seen. Cytoplasmic bridges in the form of short, thin cytoplasmic processes connecting the neighboring cells were observed. Figure

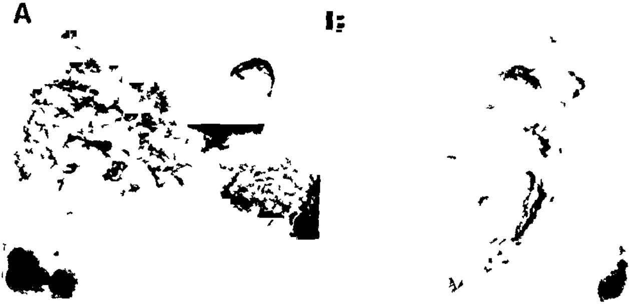

Figure 4
Malignant melanoma cells (A) Epithelial cell type A group of melanoma cells (left) covered with irregular long MV and one melanoma cell with numerous surface blebs (right) (× 3 200) Inset LM of the same cells (× 800) (B) Spindle cell type Binucleated malignant melanoma cell Note smooth surface with only occasional small blebs (× 3 700) Inset LM of the same cell (× 600)

5E and D) The cells were attached to the glass by a large veil of thin lamellar cytoplasm (Figure 5F)

Discussion

This study confirmed prior observations (for a summary see reference 14) that the great majority of cells obtained by thin needle aspiration biopsy are viable and suitable for biologic studies The present work is the first analysis of surface features of such cells The technique used in this work offers the opportunity to study the surface configuration of appropriately identified human tumor cells of various primary origins The findings suggest that the surface configuration of human cancer in tissues is not uniform and reflects the structural arrangements of cells within the tumor For instance gland forming breast cancers were represented by cancer cells with MV confined to the luminal surface Oat cell carcinoma cells formed chains similar to those seen in tissue

Human cancer cells removed from their natural setting demonstrated a variable surface configuration ranging from numerous MV blebs or ridges to almost entirely smooth surfaces MV were seen most commonly on surfaces of tumor cells from carcinoma of the breast and malignant melanoma Malignant lymphoma cells were covered with MV and/or blebs or surface ridges Clusters of oat cell carcinoma cells had some unique features The smooth-surfaced tightly packed cells displayed cellular molding and cytoplasmic bridges apparently connecting the neighboring cells

Except for oat cell carcinoma there was no specific surface configuration for any given tumor type In fact the surface configuration of tumor cells of the same primary origin varied considerably from case to case The significance of the different ways in which cancer cells of various origins attach to glass is not clear Whether these variations reflect the different biologic status and potential of tumor cells from different cases of cancers of the same primary origin remains to be determined The work now in progress on a detailed comparison of histology transmission electron microscopy and surface configuration of tumor cells may shed some light on the problem However it must be noted that most human cancer cells suspended in fluids show abundant and irregular surface MV [5]

It also must be noted that differences in surface configuration have been observed among several human malignant melanoma cell lines in tissue culture [11] The observations suggest that the surface configuration of human cancer cells may depend on the environmental conditions However confirmatory studies are clearly required

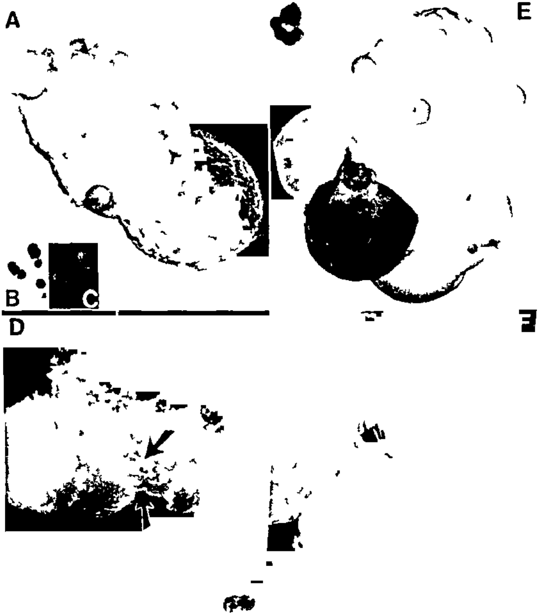

Figure 5
Oat cell carcinoma of the lung. (A) Three cancer cells. Note lack of surface MV and cytoplasmic molding (× 5,7[illegible]). (B–C) Comparable LM and SEM locating pictures for Figure 5A (× 40). (D) Two cancer cells. Note the thin intercellular cytoplasmic bridges (arrows) (× 4,[illegible]00). Inset: LM of the same cells (× 600). (E) Group of cancer cells. Note that cells are tightly packed. They have smooth surfaces and show intercellular cytoplasmic bridges (× 4,100). Inset: LM of the same group (× 600). (F) High magnification of the right part of Figure 5D. Note that the cancer cell is attached to the glass by a large thin veil of lamellar cytoplasm (× 14,000).

References

1 Berliner JA Janssen M McLatchiel The use of scanning electron microscopy in the diagnosis of malignancy in human serous effusions IITRI/SEM 11 797-802 1978

2 Domagala W Koss LG Scanning electron microscopic study *of human carcinoma and mesothelioma cells treated with cyto*chalasin B Acta Med Pol 18 301 302 1977

3 Domagala W Kahan AV Koss LG A simple method of preparation and identification of cells for scanning electron microscopy Acta Cytol 23 140-146 1979

4 Domagala W Kahan AV Koss LG The ultrastructure of surfaces of positively identified cells in the human urinary sediment A correlative light and scanning electron microscopic study Acta Cytol 23 147 155 1979

5 Domagala W Koss LG Configuration of surfaces of human cancer cells in effusions A scanning electron microscopic study of microvilli Virchows Arch [Zellpathol] 26 27-42 1977

6 Domagala W Koss LG Surface configuration of mesothelial *cells in effusions A comparative light microscopic and scan*ning electron microscopic study Virchows Arch [Zellpathol] 30 231 243 1979

7 Domagala W Woyke S Transmission and scanning electron microscopic studies of cells in effusions Acta Cytol 19 214 244 1975

8 Friedlander S A new method for detecting changes in the sur*faces of human exfoliated cervical cells with the* scanning electron microscope Acta Cytol 13 288 291 1969

9 Kaneshima S Kiyasu Y Kudo H Koga S Tanaka K An application of scanning electron microscopy to cytodiagnosis of pleural and peritoneal fluids Comparative observation of the same cells by light and scanning electron microscopy Acta Cytol 22 490-499 1978

10 Krivinkova H Ponten J Blondel T The diagnosis of cancer from body fluids A comparison of cytology DNA measurement tissue culture scanning and transmission microscopy Acta Pathol Microbiol Scand [A] 84 455-467 1976

11 Stoebner P Angusseau S Motriguard C Apport de la microscopie electronique (balayage et transmission) a l etude des liquides pleuraux non neoplastiques Arch Anat Cytol Pathol 24 135-147 1976

12 Woyke S Domagala W Czerniak B Strokowska M Fine needle aspiration cytology of malignant melanoma of the skin Acta Cytol (in press)

13 Yonda MA Aaronson SA Ellmore N Zeve VM Nagashima K *Ultrastructural studies of surface features of human normal* and tumor cells in tissue culture by scanning and transmission electron microscopy J Natl Cancer Inst 56 245-263 1976

14 Zajicek J The aspiration biopsy *In* Diagnostic Cytology and Its Histopathologic Bases Edited by LG Koss Third edition Philadelphia JB Lippincott pp 1007 1008

Cytopathology, Including Transmission and Scanning Electron Microscopy, of Pleomorphic Liposarcomas in Pleural Fluids

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Bernard Naylor M B Ch B F I A C
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Liposarcoma cells in pleural fluid from two patients with metastatic pleomorphic liposarcoma are described The major diagnostic feature of such specimens perceptible by light microscopy is the presence of solitary pleomorphic giant cells with cytoplasmic vacuoles

In some cells transmission electron microscopy revealed numerous cytoplasmic lipidic droplets With scanning electron microscopy the cellular surfaces were markedly pleomorphic with ruffles blebs and long thin processes

Liposarcomas are one of the most frequently occurring malignant neoplasms of soft tissues [2,5,14] Their major sites of metastasis include the pleura and lung [1,8,13,15,16] Yet pleural effusions caused by metastatic liposarcomas seem to be uncommon and as a result description of the cytologic manifestations of liposarcomas in serous fluids is scanty [5] We describe below two such examples including the transmission and scanning electron microscopic appearances of the liposarcoma cells from one of our cases

Materials and Methods

Smears of the sediment obtained by centrifugation of samples of the pleural fluids were wet fixed in 95% ethanol and stained by the Papanicolaou method

Samples of the buffy coat of the sediment from the pleural fluid from case 2 were removed by pipet suspended in 2% phosphate buffered glutaraldehyde and collected on a Nuclepore filter (Nuclepore Corporation Pleasanton California) with pores 3μm in diameter The filter was rinsed with buffer postfixed with 1% collidine buffered osmium tetroxide stained en bloc with 20% saturated aqueous uranyl acetate and dehydrated through a graded series of ethanol The filter was then cut into two hemicircles

The hemicircle for transmission electron microscopy (TEM) was cut into ribbons which were transferred through propylene oxide into an EPON 812-Araldite 502 mixture Thin sections were stained with Reynolds lead citrate The hemicircle for scanning electron microscopy (SEM) was critical point dried in liquid carbon dioxide and then dc sputter coated with gold

Case Histories

Case 1 The patient was a 44 year-old woman who had a pleomorphic liposarcoma of the right flank incompletely excised in February and May 1972 followed by systemic chemotherapy In July 1972 an x ray film of the chest revealed pulmonary metastatic neoplasm and bilateral pleural effusions Pleural aspirations yielded a total of 3 Liters of serosanguinous fluid which was found to contain neoplastic cells The patient died in October 1972

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Necropsy showed a fungating lesion on the right flank measuring 20×10 cm that extended into the retroperitoneum Metastatic neoplasm was present on the pleurae and in the lungs bronchial lymph nodes and thoracic soft tissues In the right pleural space 750 ml of serosanguinous fluid was present

Case 2 The patient was a 51 year-old man who underwent local excision of a pleomorphic liposarcoma from his right thigh in March 1977 Seven months later wide resection of a local recurrence was performed At the same time an x ray film of the chest revealed pulmonary metastatic neoplasm In January 1979 he was found to have a massive left pleural effusion Two aspirates yielded a total of 3 liters of serosanguinous fluid cytologic examination of the fluids revealed neoplastic cells He was discharged to be followed as an outpatient

Results

Histopathology

The neoplasm resected from the first patient was composed almost entirely of medium-sized to giant sized cells (Figures 1 and 2) The smaller cells were polygonal or fusiform The giant cells were more pleomorphic some were very large The lipoblastic nature of the neoplasm was manifested by cytoplasmic vacuolization In some cells the cytoplasm was foamy since it contained numerous tiny vacuoles whereas in others it contained larger vacuoles of various sizes Rarely signet ring cells were observed Most of the neoplastic cells however had homogeneous granular eosinophilic cytoplasm with no evidence of lipid

The cells were predominantly mononuclear The size of the nuclei varied from medium to extremely large Many were so hyperchromatic that no internal structure was visible Some of the nuclear shapes were extremely irregular Nucleoli were prominent in some nuclei Apparent intranuclear vacuoles were seen infrequently Mitotic figures were present in most microscopic fields and some were abnormal The neoplasm was very cellular without obvious stroma Focal necrosis was present

The neoplasm of the second patient was similar to that described above (Figure 3) However a higher proportion of cells contained cytoplasmic vacuoles Furthermore within the second neoplasm there were myxoid areas where the spindle and stellate cells had more vesicular nuclei

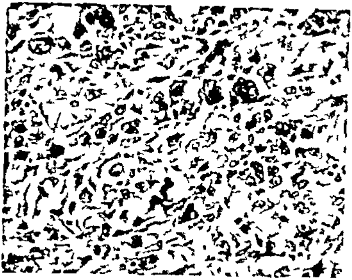

Figure 1
Case 1 Low power view of the highly cellular neoplasm demonstrates the rather haphazard arrangement of the liposarcoma cells in most of which no cytoplasmic vacuoles are discernible (hematoxylin and eosin × 208)

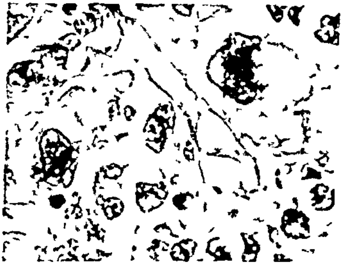

Figure 2
Case 1 Vacuoles are obvious in some of the neoplastic cells revealing their liposarcomatous nature Marked nuclear and cellular pleomorphism is evident (hematoxylin and eosin × 528)

Cytopathology Light Microscopy

In both patients the malignant nature of the pleural effusions was obviously cytologically The cellular samples contained numerous cells that were easily recognized as being neoplastic (Figures 4 to 8) Many of the cells were extremely large up to 370µm in diameter with nuclear diameters up to 300µm With rare exceptions the neoplastic cells were solitary Even within the occasional small cluster of liposarcoma cells the cells were loosely cohesive The back

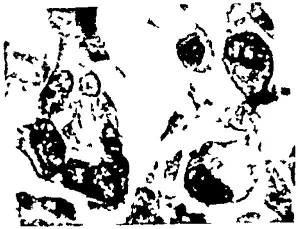

Figure 3
Case 2. Liposarcoma of thigh. The liposarcoma cells show great variation in the degree of cytoplasmic vacuolization. Although most cells are mononuclear, an occasional cell is multinucleate (hematoxylin and eosin, ×5 8)

Figure [illegible]
Case [illegible]

Figure 4
Case 1. The great d ers v of ce [illegible]
The largest cell demonstrates [illegible]
distribution of cytoplasmic vacuoles [illegible]

Figure [illegible]

unded

ground of the smears cons[illegible] erythrocytes and lymphocytes [illegible] ber of histiocytes and [illegible]

The variation in the diameter [illegible] was remarkable, ranging from 17 [illegible] Cellular and nuclear pleomorphism was striking (Figures 4 to 6). Some nuclei were round or oval (Figures [illegible] and 8), whereas others were [illegible] ures 4 and 6). There was a tendency for the [illegible] bizarre nuclei to occur in the larger cells [illegible] most of the neoplastic cells were [illegible] nucleation was commonly observed (Figures 4 to 6

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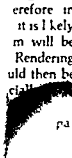

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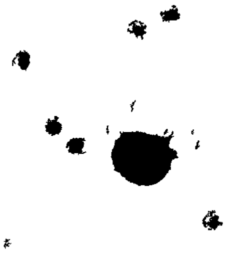

Figure 7
Case 1 This solitary liposarcoma cell possesses cytoplasmic vacuoles of various sizes and a signet ring configuration (Papanicolaou stain × 660)

Figure 8
Case 2 The nuclear membranes are quite distinct. Easily recognizable macronucleoli are present (Papanicolaou stain × 660)

was rarely observed. Mitotic figures were quite uncommon. Nuclear-cytoplasmic ratios varied considerably: in a small proportion of the liposarcoma cells, the nuclear-cytoplasmic ratio was somewhat less than 50%, whereas in others almost the entire cell was occupied by the nucleus.

While some of the sarcoma cells were dominated by a single large clear cytoplasmic vacuole, many contained numerous vacuoles of various sizes (Figures 5 and 7). In most cells the vacuoles were peripherally situated (Figures 5 and 7). Other malignant cells, apparently having lost the ability to produce lipid, exhibited no vacuoles. These cells contained homogeneous, lightly cyanophilic cytoplasm. Regardless of whether cytoplasmic vacuoles were evident, all cellular borders were indistinct (Figures 5, 6 and 8). These wispy, ill-defined edges blended imperceptibly into the background of the smear.

Cytopathology Ultrastructure

With TEM the shape of the sarcoma cells from the second patient varied greatly, from polygonal to elongated. The eccentrically located nuclei were extremely pleomorphic, ranging from round to deeply lobated (Figure 9). Occasionally, multinucleation was discerned. The heterochromatin varied in both amount and distribution. Some nuclei were nearly filled with large clumps, whereas others had only a thin rim along the nuclear membrane. Huge, compact nucleoli were seen in some nuclei. Most cells had abundant cytoplasm containing large numbers of flat profiles of rough endoplasmic reticulum scattered *throughout the cell without an organized pattern*. Golgi complexes were small and well organized. Small amounts of smooth endoplasmic reticulum with vesicular profiles were present in many cells. The mitochondria were small and few in number. Their lamellar cristae were often oriented along the long axis of the organelle. Microfilaments were scattered through the cytoplasm but were most conspicuous where there were few polyribosomes. However, they did not form a distinctive perinuclear zone. Most cells contained cytoplasmic lipidic droplets

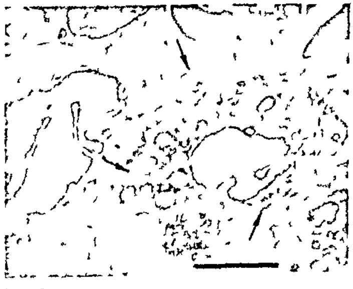

Figure 9
TEM of two liposarcoma cells, one containing numerous lipidic droplets (arrows). Mark is 5μm

which varied greatly in size A few contained scattered glycogen particles within the cytoplasmic matrix (Figure 10) The contours of the cell surfaces were highly irregular with unusual thin cell processes resembling microvilli Specific intercellular junctions were not observed Pinocytosis was not evident

The malignant cells seen by SEM appeared quite pleomorphic They occurred individually and were ovoid or ellipsoid (Figures 11 and 12) Stellate or fusiform cells were not seen Cell surface structure varied considerably Small ruffles various-sized blebs and irregular microvilli were all noted on the sarcoma cells The neoplastic cells were mixed with erythrocytes lymphocytes histiocytes and a considerable amount of fibrin

Discussion

Although the lungs and pleurae are among the most common sites of metastatic liposarcomas [8,11,13,16] the frequency of malignant pleural effusions due to these neoplasms is not recorded The recent literature contains a number of excellent reviews of liposarcomas but none mentions the associated serous effusions [1,2,8,11,12,15,1]

Morton and his colleagues[10] performed viral studies in tissue culture on liposarcoma cells obtained from pleural fluid However their report did not deal with the cytomorphology of such fluids Such descriptions have been virtually limited to those of Hajdu and Hajdu [3] Their book contained several illustrations of exfoliated cells from liposarcomas one derived from pleural fluid Yet they did not emphasize the marked pleomorphism and massive size attainable by the neoplastic cells these were prominent features of the cells in our material

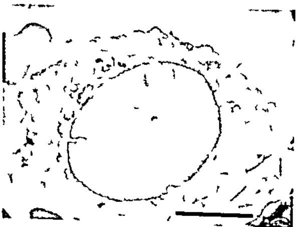

Figure 10
TEM of a poorly differentiated liposarcoma cell Mark is 5μm

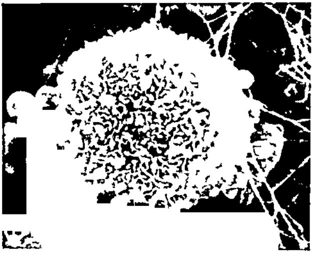

Figure 11
SEM of liposarcoma cell covered with numerous ruffles and blebs Marks are 5μm

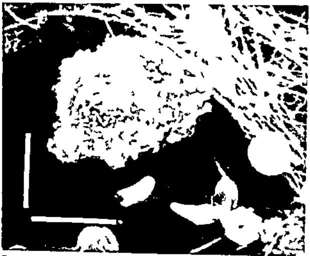

Figure 12
SEM of liposarcoma cell attached to fibrin mesh and surrounded by erythrocytes Marks are 5μm

Liposarcomas most often present as a large mass on a limb or in the retroperitoneum Therefore in most patients who harbor such neoplasms it is likely that the nature of the primary neoplasm will be known before a serous effusion develops Rendering a cytologic diagnosis of liposarcoma would then be relatively easy Yet in some patients especially those with an abdominal liposarcoma the true nature of the lesion may not be recognized early In these pa

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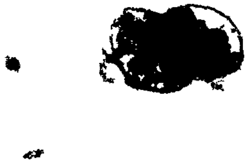

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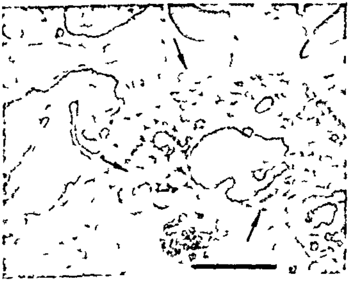

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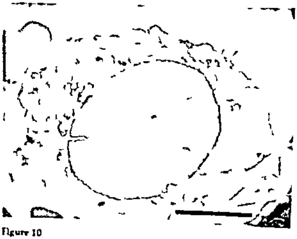
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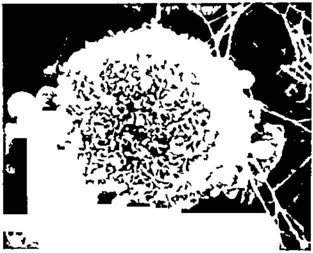
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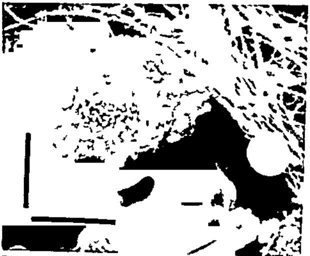
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Several morphologic features may be helpful in discriminating between metastatic liposarcomas and adenocarcinomas First the enormous size of some of the cells in the pleomorphic *liposarcomas is rarely attained by adenocarcinomas* Usually cells of adenocarcinomas are not as pleomorphic as those of pleomorphic liposarcomas The degree of vacuolization is greater in many of the cells of the pleomorphic liposarcomas than that in typical adenocarcinoma cells Adenocarcinoma cells often are well delimited with distinct cell boundaries whereas the edges of liposarcoma cells are ill defined and appear to blend into the background Finally a major difference was the almost total lack of cohesiveness of the liposarcoma cells from both of our patients Even in those adenocarcinomas characterized mainly by solitary cells some cell clusters are usually apparent

The absence of cohesiveness may help to distinguish the cells of pleomorphic liposarcomas from those of other malignant mesenchymal tumors Hajdu and Hajdu[5] included synovial sarcomas and angiosarcomas within the differential diagnosis According to these authors the cells from both of these neoplasms show a tendency to cluster The extreme pleomorphism of these liposarcomas may also be a clue to their identity Bizarre giant cells may be a feature of certain other sarcomas such as certain rhabdomyosarcomas [5] However the presence of hyperchromatic nuclei within such giant cells may suggest a liposarcoma [5,16] Finally marked cytoplasmic vacuolization is also suggestive of liposarcoma

TEM is also helpful in separating pleomorphic liposarcomas from other malignant neoplasms The absence of specific intercellular junctions aids in eliminating carcinomas from *consideration More definitively* the presence of well-developed lipid-containing organelles confirms the diagnosis of liposarcoma The first reported ultrastructural study of a pleomorphic liposarcoma was that by Kalderon and Fethiere[6] recently Reddick et al summarized their findings in three such neoplasms [12] This second group of workers claimed that pleomorphic liposarcomas can be distinguished from malignant fibrous histiocytomas on the basis of a conspicuous perinuclear zone of microfilaments in the latter neoplasm absent in the former and from pleomorphic rhabdomyosarcomas by the lack of myosin filaments in liposarcomas The ultrastructure of other classes of liposarcomas has been reported infrequently [3,4,6,11,14]

We are unaware of any previous report of a scanning electron microscopic study of a liposarcoma Our work demonstrated the marked pleomorphism of these malignant cells it also revealed the bizarre *and highly variable nature of their surfaces* Adenocarcinoma cells in effusions could be easily distinguished from liposarcoma by SEM However it would not help to distinguish small liposarcoma cells from histiocytes which have similar surface characteristics [7]

References

1 Enterline HT Culberson JD Rochlin DB Brady LW Liposarcoma A clinical and pathological study of 53 cases Cancer 13 932 950 1960

2 Enzinger FM Winslow DJ Liposarcoma A study of 103 cases Virchows Arch [Pathol Anat] 335 367 388 1962

3 Feldman PS A comparative study including ultrastructure of intramuscular myxoma and myxoid liposarcoma Cancer 43 512 525 1979

4 Flenker H Myxoid liposarcoma Light and electron microscopic investigation Virchows Arch [Pathol Anat] 371 171 176 1976

5 Hajdu SI Hajdu EO Cytopathology of Sarcomas and Other Non Epithelial Malignant Tumors Philadelphia WB Saunders 1976 pp 224 225 243-247

6 Kalderon AE Fethiere W Fine structure of two liposarcomas Lab Invest 28 60-69 1973

7 Kaneshima S Kiyasu Y Kudo H Koga S *Tanaka K An application* of scanning electron microscopy to cytodiagnosis of pleural and peritoneal fluids Comparative observations of the same cells by light microscopy and scanning electron microscopy Acta Cytol 22 490-499 1978

8 Kindblom LG Angervall L Svendsen P Liposarcoma A clinicopathologic radiologic and prognostic study Acta Pathol Microbiol Scand [A] Suppl 253 1 71 1975

9 Kinne DW Chu FCH Huvos AG Yagoda A Fortner JG Treatment of primary and recurrent retroperitoneal liposarcoma Twenty-five year experience at Memorial *Hospital* Cancer 31 53 56 1973

10 Morton DL Hall WT Malmgren RA Human liposarcoma Tissue cultures containing foci of transformed cells with viral particles Science 165 813-816 1969

11 Razzuk MA *Urschel HC Jr Race GJ* Kingsley WB Paulson DL Liposarcoma of the mediastinum Case report and review of the literature J Thorac Cardiovasc Surg 61 819 826 1971

12 Reddick RL Michelitch H Triche TJ Malignant soft tissue tumors (malignant fibrous histiocytoma pleomorphic liposar*coma and pleomorphic rhabdomyosarcoma) An electron mi*croscopic study Hum Pathol 10 327 343 1979

13 Reszel PA Soule EH Coventry MB Liposarcoma of the extremities and limb girdles A study of two hundred twenty two cases J Bone Joint Surg 48 A 229 244 1966

14 Scarpelli DG Greider MH A correlative cytochemical and electron microscopic study of a liposarcoma Cancer 15 776-789 1962

15 Spittle ME Newton KA Mackenzie DH Liposarcoma A review of 60 cases Br J Cancer 24 696-704 1970

16 Stout AI Lattes R Liposarcoma *In* Tumors of the Soft Tissues Atlas of Tumor Pathology Second series fascicle 1 Washington DC Armed Forces Institute of Pathology 1967 pp 116 126

American Society of Cytology

The American Society of Cytology will hold its 28th *Annual Scientific Meeting in Boston Massachusetts No-*
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The Potential Value of Immunoperoxidase Techniques in Diagnostic Cytology

Mehrdad Nadji, M D , M I A C

A slightly modified immunoperoxidase method was developed in our laboratory and applied to a variety of aspiration and exfoliative cytologic material Our aims were (1) to explore the applicability of the immunoperoxidase procedure to diagnostic cytology (2) to attempt to define the histogenesis of neoplastic cells when morphology alone proved insufficient and (3) to investigate the possibility of differentiating reactive from neoplastic lymphoreticular disorders by studying their immunoglobulin patterns

Our findings indicate that the immunoperoxidase technique is applicable to cytologic material The simplicity of the procedure combined with its high sensitivity and excellent morphology merits wider application of this technique to routine diagnostic cytology

Immunoperoxidase techniques are a group of immunoenzymatic procedures capable of demonstrating various cell and tissue antigens They share many principles with the already established immunofluorescence methods Immunoperoxidase however lacks the major disadvantages of immunofluorescence namely, instability of stain requirement for fresh tissue and specialized microscopy These combined with the apparently higher sensitivity of the technique and its superior morphologic results are responsible for the increasing popularity of immunoperoxidase techniques and are reflected in the rapid proliferation of reports on applications of the method in the recent medical literature

With few exceptions however the application of immunoperoxidase procedures in diagnostic pathology has been limited to its utilization in histopathology Cytology surprisingly has enjoyed very little of the potential value of these techniques as an aid to histogenetic diagnosis This report is the result of our experience with the application of immunoperoxidase procedures to routine cytologic material in the past few years

Materials and Methods

Source of Specimens

Routine cytologic specimens were utilized primarily in this study These included cervicovaginal exfoliative material serous fluids brush and wash specimens fine needle aspirations and tissue imprints The majority of cell samples studied however were those from body cavity effusions and fine needle aspirations

Types of Preparation

Cell smears imprints and cell blocks as well as filter and cytocentrifuge preparations were all utilized for the immunoperoxidase staining Equally good results were obtained from all types of cell preparations with the exception of filter specimens The heavy nonspecific background staining of the filter masked the underlying cellular reaction and therefore in our hands filter preparations were found unsuitable for the evaluation of immunoperoxidase reactions in cytology Cell block preparations had to be deparaffinized prior to the immunologic procedure hence they were handled in a manner similar to that for the histopathologic specimens

Types of Fixation

The immunoperoxidase procedure was carried out on

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cell samples fixed by a variety of techniques Best results however were obtained from the preparations fixed either in 95% ethanol or buffered formol acetone The latter was suggested by Mason et al[9] who studied the effects of different cell fixatives on the results of immunoperoxidase staining of peripheral blood and bone marrow smears

On many occasions the consideration for immunoperoxidase study came about only after examination of the Papanicolaou-stained preparations In these instances the coverglass was removed and the specimens were destained in a solution of acid alcohol and then carried through the steps of the immunologic reaction

Immunoperoxidase Techniques

In each of the several variations of the immunoperoxidase method the enzymatic activity of horseradish peroxidase is used in the presence of hydrogen peroxide and a chromogenic substrate e g diaminobenzidine as a visual marker Most of the currently utilized techniques fall into two general categories peroxidase conjugate methods and unlabelled antibody procedures In our laboratory we prefer the unlabelled antibody peroxidase antiperoxidase (PAP) procedure pioneered by Sternberger[14] Among the advantages of the PAP technique are its higher sensitivity and the availability of good-quality commercial PAP preparations For details of theoretical and technical aspects of PAP and other immunoperoxidase procedures the reader should refer to the excellent recent reviews by Sternberger[14] and *Taylor*[3]

PAP Staining Procedure

The following is the step by-step sequence of the PAP method for cytologic specimens as performed in our laboratory

1 Circle the specimen on the glass slide with a diamond marker

2 Deparaffinize in xylol 3 minutes (for cell blocks)

3 Hydrate in decreasing grades of ethanol

4 Block endogenous peroxidase with 0 3% *hydrogen* peroxide in methanol 30 minutes

5 Rinse in tap water and wash in phosphate-buffered saline (PBS) 5 minutes

6 Cover the circles with normal swine serum (1 20 30 minutes) to reduce nonspecific background staining

7 Decant excess normal swine serum and add drops of rabbit antiserum *to the antigen under study* Proper dilution of primary rabbit antiserum should be determined by checkerboard study

8 Wash with three changes of PBS 10 minutes each

9 Add drops of swine antirabbit immunoglobulin 1 20 30 minutes

10 Wash with three changes of PBS

11 Add drops of peroxidase antiperoxidase (rabbit) proper dilution should be determined from checkerboard study 30 minutes

12 Wash with three changes of PBS

13 Add drops of 3 3 diaminobenzide tetrahydrochloride (DAB) solution containing hydrogen peroxidase (6 mg DAB + 10 ml PBS + 4 drops of 3% hydrogen peroxide) 3 to 8 minutes This solution should be prepared fresh before administration While working with DAB one should use caution and wear a mask and gloves The DAB reaction should preferably be carried out under a ventilated hood

14 Wash with running tap water 5 minutes

15 Counterstain with hematoxylin or other nuclear stains

16 Dehydrate with increasing grades of ethanol clear in xylol and mount

Swine antirabbit immunoglobulin and rabbit PAP were obtained commercially (Dako Accurate Chemical and Scientific Corporation Hicksville New York) The following primary antisera were used in this study rabbit antisera to light and heavy chain immunoglobulins and muramidase (Dako Accurate Chemical and Scientific Corporation Hicksville New York) antisera to hormones (Calbiochem Behring Corporation San Diego California) antibodies to oncodevelopmental antigens (Dr A Castro Hormone Research Laboratory University of Miami School of Medicine Miami Florida) and rabbit antiserum to prostate-specific acid phosphatase (generously supplied by Dr T M Chu Roswell Park Memorial Institute Buffalo New York) All dilutions were made by phosphate-buffered saline *(pH 7 4 to 7 6) All* reactions were performed at room temperature (22 C) using a *humidity chamber* to reduce evaporation of the reagants *The actual* duration of incubation in step 7 varied considerably depending on the type and *the strength of the* primary rabbit antiserum *Although* the majority of reactions *took* place in 30 minutes with some antisera particularly *those developed for* use in *radioimmuno*assay procedures incubation times of 18 to 48 hours at 4 C were *required*

Optimal dilution of primary rabbit *antiserum* was calculated by checkerboard studies In histopathol ogy this can be accomplished by testing serial dilutions of primary antiserum against several concentrations of PAP on multiple recuts from the same tissue block In cytology however the number of available slides may be a limiting factor We have used multiple imprints of normal tissues e g spleen for histiocytes in order to overcome this problem Whenever this was not feasible concentrations of antibody slightly higher than those which proved optimal for tissue section were used successfully

Controls

Since truly monospecific antibodies probably do not exist it is imperative that known positives and negatives are included in each immunoperoxidase reaction Every run should also include antibody controls by omitting the primary antibody and/or substituting the primary antiserum with nonimmune rabbit serum On those occasions in which only one slide was available for study we utilized one-half of the slide surface for the antibody control Two circles were etched on the slide using a diamond marker One circle was covered by primary antiserum, while nonimmune serum was added to the adjacent circle

Results

Interpretation

Positive reactions appear as brown granules contained within the cell or on the surface of cell membranes depending on the location of the antigens under study Frequently in cytologic material from fine needle aspiration or body cavity fluids a positive reaction in the background material may be encountered as well This is not surprising since intracellular antigens may be either secreted by the cells or extruded from the cell by the mechanical action of smearing the cells on the slide

For the positive results to be considered true and valid the known positive slide should be positive whereas known negative and antibody controls should show a negative reaction Intracellular hemosiderin melanin and similar pigments are different from DAB in color and texture Examination of control slides will also show their presence Finally degenerated and necrotic cells often show a nonspecific positive reaction For this reason positive interpretation should be limited to those cells which appear viable and morphologically intact

Application

Immunoperoxidase procedures in this study were primarily utilized to demonstrate intracellular antigens These included immunoglobulins (light and heavy chains) enzymes (muramidase and prostate-specific acid phophatase) hormones (gastrin calcitonin insulin and glucagon) and tumor markers (α fetoprotein human chorionic gonadotropin and carcinoembryonic antigen)

Immunoglobulins

A variety of lymphoproliferative disorders were studied for evidence of monoclonal immunoglobulin production utilizing anti κ and anti λ light chain antibodies The monoclonality and therefore the neoplastic nature of five samples (two myelomas and three large cell lymphomas) were clearly demonstrated by this technique (Figures 1 to 3) Conversely 11 nonneoplastic lesions including two cases of chronic lymphocytic thyroiditis and four aspirations from reactive lymph nodes depicted a polyclonal pattern of intracellular immunoglobulins

It is important to note that the method was found unsuitable for demonstration of immunoglobulins in small lymphocytes For example tuberculous pleuritis and a pleural effusion in a case of chronic lymphocytic leukemia could not be distinguished since both samples contained predominantly well-differentiated small lymphocytes None of the 14 nonlymphoid malignancies showed intracellular immunoglobulins

Enzymes

The value of prostatic acid phosphatase as an immunohistochemical marker to differentiate prostatic from nonprostatic neoplasms has been established by us previously [11] The same antiserum was used to confirm the prostatic origin of three adenocarcinomas on fine needle aspirations from lymph nodes (Figure 4) Prostatic primary was ruled out on four other samples (two aspirates and two fluids) utilizing the same technique All positive and negative cases were subsequently confirmed by histologic and/or clinical findings

Cytoplasmic muramidase (lysozyme) was demonstrated in histiocytes and macrophages whereas lymphocytes stained consistently negative The intensity of the reaction varied from cell to cell but it was more striking in cells actively involved in phagocytosis Pulmonary macrophages polymorphonuclear leukocytes and occasional reactive mesothelial cells showed positive staining (Figure 5)

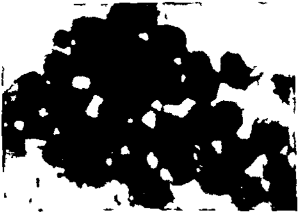

Figure 1
Monoclonal staining of neoplastic plasma cells for κ light chain in pleural effusion (cytocentrifuge preparation PAP-hematoxylin × 500)

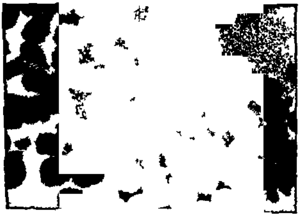

Figure 2
Same case as Figure 1 negative reaction for λ light chain (cytocentrifuge preparation PAP-hematoxylin × 500)

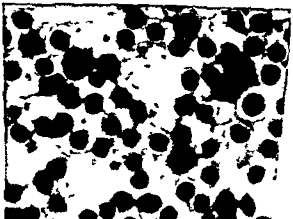

Figure 3
Malignant lymphoma Monoclonal reaction for κ light chain [illegible] needle aspiration of pelvic mass PAP-hematoxylin × 500

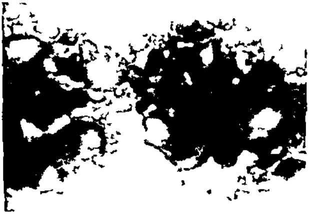

Figure 4
Prostatic carcinoma Positive reaction for prostate-specific acid phosphatase (fine needle aspiration of cervical lymph node PAP-hematoxylin × 500)

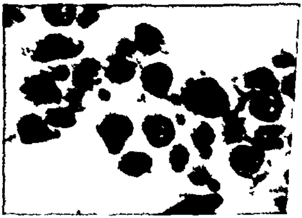

Figure 5
Pulmonary macrophages Positive reaction for muramidase (cell block preparation from bronchial washings PAP-hematoxylin × 500)

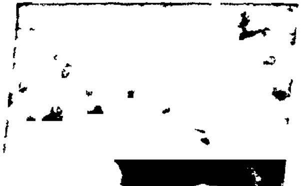

Figure 6
[illegible] Positive reaction for human [illegible] [illegible] PAP-hematoxylin × 500)

Hormones

Intracellular gastrin was demonstrated in an aspirate from a pancreatic islet cell tumor The patient had clinical symptoms of the Zollinger Ellison syndrome Subsequent autopsy revealed widespread tumor with multiple hepatic metastases

Tissue imprints from two other pancreatic islet cell tumors which clinically appeared nonfunctional showed positive reaction for gastrin insulin and glucagon concomitantly These cases required 24 hours incubation with the primary antiserum for optimum results An imprint from a medullary carcinoma of the thyroid gland was similarly positively stained for calcitonin

Oncodevelopmental Antigens

The β subunit of human chorionic gonadotropin was localized in benign syncytiotrophoblasts on cervical smears of two pregnant females with first trimester bleeding (Figure 6) Giant cell histiocytes and multinucleated decidual cells in several other samples showed a negative reaction for chorionic gonadotropin Malignant trophoblasts on a fine needle aspiration sample from a patient with metastatic mixed germ cell tumor of the ovary in the lung exhibited a variable but positive reaction

Carcinoembryonic antigen was localized intracytoplasmically in the following samples fine needle aspiration of the liver with metastatic signet ring cell carcinoma of the stomach cells from primary adenocarcinoma of the lung in pleural fluid and cells from mucinous cystadenocarcinoma of the ovary in peritoneal fluid

α fetoprotein was demonstrated both in tissue and cells of ascitic fluid from a young patient with recurrent endodermal sinus tumor of the ovary

Discussion

The morphologic criteria currently used in diagnostic cytology at times fail to define the true nature or histogenetic origin of neoplastic cells Ancillary means such as scanning or transmission electron microscopy cell culture and chromosomal studies have been used sporadically as an aid to specific identification of tumor cells However these techniques fall short of being practical for daily usage in cytology are considered a luxury and are not available in many laboratories

Special cytochemical stains e g mucicarmine or colloidal iron have occasionally aided the cytologist but these tinctorial reactions are often nonspecific and reflect the underlying cellular metabolism which is shared by a variety of different cell types

When the immunofluorescence technique was introduced in 1941 [4] it was hoped that it would become a routine procedure in pathology providing important information about the function or histogenesis of various lesions The requirement of fresh or specially prepared tissue coupled with complex ultraviolet microscopy and poor resolution of cell or tissue morphology severely limited the widespread use of this method

In 1966 enzyme labelled antibodies were developed[1,12] and have been successfully applied to formalin fixed paraffin-embedded routine histopathologic sections [3,5,17] Immunoperoxidase techniques have added the long awaited new dimension to pathology by combining excellent morphology with specific demonstration of cellular antigens Due to the versatility of the technique it became possible for the first time to study histopathologic material in a retrospective fashion [15] During the first decade following the introduction of immunoperoxidase the technique was primarily applied to histopathologic material Limited experience has been reported with regard to its usage in diagnostic cytology

Many cellular antigens withstand conventional preparation fixation and staining methods In cytologic samples although the cell membrane is not mechanically opened as it is with histologic sections there appears to be adequate penetration of antisera into the cells following many routine fixation techniques When combined with excellent cytomorphologic preservation this makes immunoperoxidase an ideal special stain in diagnostic cytopathology

In this paper several examples of the potential value of the immunoperoxidase technique in diagnostic cytology are presented For example akin to histopathology positive cytoplasmic staining for immunoglobulins may distinguish malignant lymphomas from undifferentiated epithelial or mesenchymal malignancies A monoclonal reaction for immunoglobulin can be used as an indication of the neoplastic nature of an otherwise ambiguous lymphoreticular lesion Similarly identification and differentiation of various endocrine neoplasms can be aided by demonstration of their cytoplasmic hormonal content

Undoubtedly characterization of different cell lines by immunochemical techniques utilizing cell specific antisera will play a major role in the future of routine diagnostic histopathology as well as cytology Several of these specific cell markers are already being utilized in a number of laboratories The following is a partial listing of some of these cell specific products already in use muramidase for histiocytes monocytes and macrophages[10] prostatic acid phosphatase for benign or malignant prostatic glandular epithelium[7,11] factor VIII related antigen for endothelial cells[2] glial fibrillary acidic protein for benign or malignant glial cells[6] and antimesothelial cell serum for benign and malignant mesothelial cells [13] For a complete listing of potential diagnostic markers the reader should refer to the excellent review article by DeLellis et al [5]

The importance of strict adherence to the usage of controls as a basis for the critical appraisal of the results cannot be overemphasized All too often the commercially available antisera are not truly monospecific and absorption studies are necessary in order to validate a positive reaction Similar to other immunologic reactions with immunoperoxidase techniques the danger of false negative and false positive results should be circumvented by the simultaneous study of known positive and negative control cases

It is hoped that as more truly monospecific antisera (produced by hybridomas) become available immunoperoxidase methods will be used more often as additional diagnostic tools to complement traditional cytomorphologic criteria

Acknowlegments

The secretarial expertise of Deborah Cunningham is greatly appreciated

References

1 Avrameas S Uriel J Méthode de marquage d antigenes et d anticorps avec des enzymes et son application en immunodiffusion C R Acad Sci [D] 6 2543 2545 1966

Bloom AL The biosynthesis of factor VIII Clin Haematol 8 53 [illegible] 1979

3 Bosman FT Nieuwenhuijzen Kruseman AC Clinical applications of the enzyme labeled antibody method Immunoperoxidase methods in diagnostic histopathology J Histochem Cytochem 1140-1147 1979

4 Coons AH Creech JH Jones RN Immunologic properties of an antibody containing a fluorescent group Proc Soc Exp Biol 47 200-202 1941

5 DeLellis RA Sternberger LA Mann RB Banks PM Nakane PK Immunoperoxdase technics in diagnostic pathology Report of a workshop sponsored by the National Cancer Institute Am J Clin Pathol 71 483-488 1979

6 Eng LF Rubinstein LJ Contributions of immunohistochemistry to diagnostic problems of human cerebral tumors J Histochem Cytochem 26 513-522 1978

7 Jobsis AC DeVries GP Anholt RRH Sanders GTB Demonstration of the prostatic origin of metastases An immunohistochemical method for formalin-fixed embedded tissue Cancer 41 1788 1793 1978

8 Kohler G Milstein C Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion Eur J Immunol 6 511 519 1976

9 Mason DY Farrell C Taylor CR The detection of intracellular antigens in human leukocytes by immunoperoxidase staining Br J Haematol 31 361 370 1975

10 Mason DY Taylor CR The distribution of muramidase (lysozyme) in human tissues J Clin Pathol 28 124 131 1975

11 Nadji M Tabei SZ Castro A Chu TM Morales AR Prostatic origin of tumors An immunohistochemical study Am J Clin Pathol 73 735 739 1980

12 Nakane PK Pierce GB Jr Enzyme-labeled antibodies Preparation and application for the localization of antigens J Histochem Cytochem 14 929-931 1966

13 Singh G Whiteside TL Dekker A Immunodiagnosis of mesothelioma Use of antimesothelial cell serum in an indirect immunofluorescence assay Cancer 43 2 88 2296 1979

14 Sternberger LA Immunocytochemistry Second edition New York John Wiley & Sons 1979 pp 1 354

15 Taylor CR Immunohistological approach to tumor diagnosis Oncology 35 189 197 1978

16 Taylor CR Immunoperoxidase techniques Practical and theoretical aspects Arch Pathol Lab Med 10 113 121 19 8

17 Taylor CR Burns J The demonstration of plasma cells and other immunoglobulin containing cells in formalin fixed paraffin-embedded tissues using peroxidase labelled antibody J Clin Path l27 14 20 1974

Immunoperoxidase Staining of Trichomonas vaginalis *in Cytologic Material*

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C M I A C
William A Gardner, Jr , M D
Betsy D Bennett M D , Ph D

In the course of studies on the histopathology and pathogenesis of trichomonal infections specific antisera to Trichomonas vaginalis *were produced Using these antisera conventional immunocytochemical techniques were modified and applied to a variety of cytologic specimens With this methodology trichomonal organisms were identified in specimens from usual and unexpected locations These findings plus the potential applications of similar methodologies to other areas of cytopathology are reported*

In recent years the literature has been inundated with reports of applications of immunocytochemical techniques to histopathology The remarkable popularity of these techniques relates to several factors (1) the specific localization and identification of antigenic sites (2) the use of tissue that has been fixed and processed by routine procedures and (3) the applicability to a wide variety of tissues and antigens Although immunocytochemical techniques represent a burgeoning area of both diagnostic and investigative histopathology we are not aware of the similar application of these techniques to diagnostic cytology This report represents our initial efforts to employ such techniques in a single discrete area of diagnostic exfoliative cytology

Trichomonas vaginalis is a common pathogen of the female genitourinary tract and can usually be identified with ease in routine Papanicolaou-stained cytologic preparations of gynecologic material In these preparations they appear as poorly defined greyish masses often not very different in staining characteristics from the background In a wet preparation such as fresh unstained urine *T vaginalis* organisms are identified largely by their motility In stained preparations they may be recognized by their shape their size the eye appearance of the nucleus and occasionally a flagellum (Figure 1) Frequently the diagnosis of this protozoan is made not so much by the morphologic identification of the organisms themselves as by the features that are traditionally associated with trichomonal infection such as perinuclear halos and 'BB shot of polymorphonuclear leukocytes [1]

The unequivocal identification of these organisms from unusual i e extragenital sites may be extremely difficult however Although trichomonads may be stained by a variety of stains including Giemsa methenamine silver alcian blue and iron hematoxylin all of these stains lack specificity and fail to differentiate the organisms from other cellular elements and the surrounding background Utilizing the antigenic specificity of trichomonal organisms immunoperoxidase staining has been employed for their specific identification This method gives reproducible results and a permanent preparation and utilizes ordinary light microscopy

Materials and Methods

Details of the preparation of the antitrichomonal antibody have been reported previously [2] The method of indirect immunoperoxidase staining em

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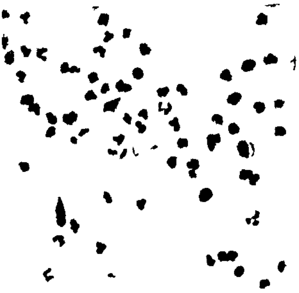


Figure 1
Cervical smear of Trichomonad with the eye appearance of the nucleus (Papanicolaou stain × 400)

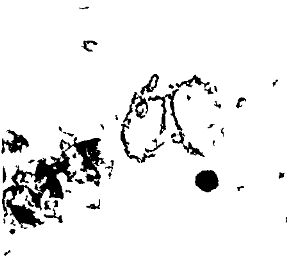


Figure 2
Trichomonas vaginalis in cervical smear after immunoperoxidase staining (× 400)

ployed is as follows (1) 0 3% H_2O_2 in methanol 30 minutes (2) 3% H_2O_2 5 minutes (3) 10% egg albumin 30 minutes (4) normal goat serum (undiluted) 10 minutes (5) antitrichomonal antibody (rabbit) 1 200 60 minutes (6) peroxidase-conjugated goat antirabbit gamma globulin 1 50 30 minutes (7) diaminobenzidine 4 minutes All solutions were prepared in Tris buffer and Tris buffered saline was used to rinse the preparations between each step

In the present study duplicate cervical smears were obtained from 100 patients seen at the Vanderbilt University Medical Center gynecology clinic One set of smears was routinely stained by the Papanicolaou method and screened The duplicate set was stained for *Trichomonas* with the indirect immunoperoxidase technique and then counterstained with either routine Papanicolaou stain or light green In addition 22 urine cytology specimens from VA patients who evidenced *Trichomonas* on routine urinalysis were also stained with this method Smears of organisms obtained directly from the culture medium were prepared and used as controls

The immunoperoxidase staining was also attempted on slides from ten cervical smears that had been previously stained by the Papanicolaou method The only additional step was the exposure of the slides to xylene to remove the coverslips

Results

The results of the application of immunoperoxidase staining to routine cervical smears are demonstrated in Figure 2 The organisms stain dark brown have a granular appearance and stand out sharply against the background They are easily noticed on low power examination especially with the light green counterstain

In order to evaluate the usefulness of this technique for identifying *Trichomonas* the results of the routine cytologic examination of the 100 cervical smears were obtained and compared with those obtained after immunoperoxidase staining *Trichomonas* organisms had been observed and reported in four cases by use of the routine preparation Using the immunoperoxidase procedure organisms were unequivocally identified in these same 4 cases and in an additional 16 Five more specimens showed what were felt to be fragmented or deformed organisms but because no classical forms were identified in these cases they are not included as positive results Diligent retrospective rescreening of the original routinely prepared slides from the 16 additional positive cases found by immunoperoxidase staining revealed the presence of probable *Trichomonas* in several cases Most of these slides however did not contain any recognizable organisms The imprecise terms *several* and *probable* are used to underscore the fact that the study was not undertaken to derive statistical comparisons but to illustrate the overall utility

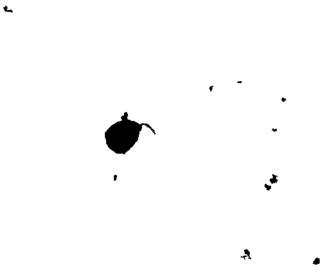


Figure 3
Trichomonas vaginalis in urine after immunoperoxidase staining Note the antibody complexing with the flagellum (× 250)

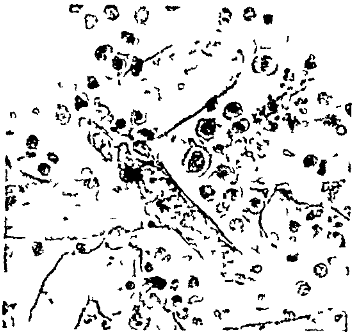


Figure 4
Urinary cast after immunoperoxidase staining Note the absence of dark brown granular structures (× 250)

of the technique To further illustrate the usefulness of this procedure we examined 22 urine cytology preparations from patients previously demonstrated to have *Trichomonas* on routine urinalysis Organisms were demonstrated in all 22 cases (Figure 3)

In the cervical smears that had been previously Papanicolaou stained no difficulty was encountered in overlaying the peroxidase stain with acceptable results The organisms were again readily identified but with the darker background in these prestained slides there was not the same degree of contrast as seen in the above cases

Discussion

Although it is a relatively new technique immunoperoxidase staining has become a very popular tool for demonstration of large numbers of different antigens in tissue sections Similar applications have been described for the recognition of several microorganisms that may be difficult to identify e g *Chlamydia*[7] and *Treponema pallidum*[1] This paper has demonstrated that the technique is also applicable to cytologic preparations The procedure is not difficult and while more time consuming than routine staining is a superior method for demonstration of *Trichomonas* organisms in cervical smears This finding is certainly of epidemiologic interest and probably of clinical interest as well

Perhaps the major clinical usefulness of this technique however relates to the examination of material from unusual sites Figure 4 shows a urine cytology with a castlike structure containing several indistinct structures some of which contain an eye suggestive of *Trichomonas* This would be an extremely unlikely finding since the cast structure would indicate the presence of organisms within the kidney itself Immunoperoxidase staining of the cast was in fact negative confirming that these structures were not trichomonal organisms A similar situation was noted in a cytology preparation from fluid obtained from a renal cyst (Figure 5A) The cells with a vague outline and grey blue cytoplasm (arrows) were noted by the screening cytotechnologists to bear some resemblance to *Trichomonas* Immunoperoxidase staining of the same preparation unequivocally established that these structures were indeed *Trichomonas* (Figure 5B)—again an unlikely diagnosis but on in which this technique provided the final proof

Trichomonas has been previously reported in various extragenital sites including sputum[4] pleural fluid[3] and retroperitoneal tissue[6] The diagnosis of organisms in these areas has been made by methods ranging from culture to scanning electron microscopy to a consensus opinion of microbiologists at the Center for Disease Control Most authors in fact have commented on the difficulty of proving the occurrence of trichomonads on microscope slides

Immunoperoxidase staining represents a relatively easy and rapid approach to the unequivocal diagnosis of *Trichomonas* infestation regardless of the loca

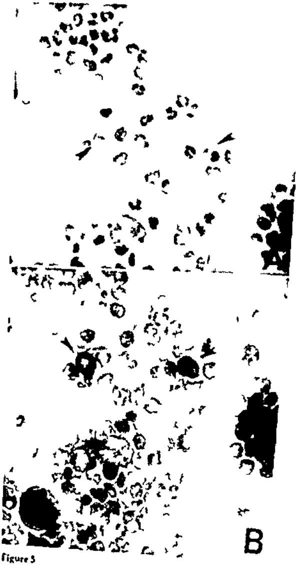


Figure 5

(A) Papanicolaou-stained renal cyst fluid Arrows [illegible] T [illegible] organisms (X [illegible]50) (B) Renal cyst fluid af[illegible] immunoper[illegible]oxidase staining Trich[illegible]monas (arrows) appear as dark and granular structures (X [illegible]50

tion or state of preservation of the [illegible] vious staining of the prepara[illegible] [illegible] seem to have application not only for the [illegible] case but also for the more accura[illegible] of the epidemiology of this very [illegible]

Acknowledgements

We are indebted to Richard J Da[illegible] [illegible] taining the cervical smears [illegible] Linda V Kelly C T (ASCP) [illegible] the urine slides

References

1 Beckett JH Bigbee JW Immunoperoxidase [illegible] onema pallidum Arch Pa[illegible] La[illegible] Med [illegible]

2 Bennett BD Bailey JM Gardner WA Immuno[illegible] identification of trichomonads Arch Patho[illegible] 249 1980

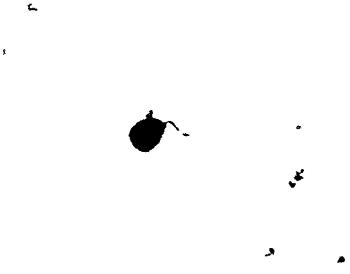
3 Memik F Trich[illegible] [illegible] 212 1968

4 [illegible]gaki G Benirschke K [illegible] granuloma of the pelvic cavity [illegible] 15 679-681 19[illegible]8

5 Naib Z Exfoliative Cytopathology [illegible] Little Brown 19[illegible] pp [illegible]

6 Walton BC Bacharach T Occurrence [illegible] respiratory tract Report of three cases [illegible] 1963

7 Woodland RM El Sheikh H Da[illegible] of immunoperoxidase and [illegible] tecting Chlamydia in conjunct[illegible] [illegible] J Clin Pathol 31 1073 107[illegible] 1[illegible]



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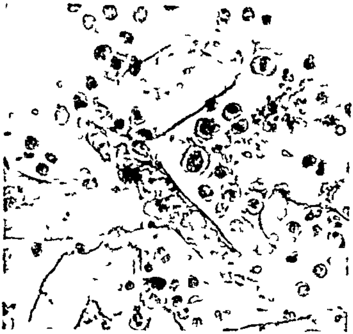
Trichomonas vaginalis in urine after immunoperoxidase staining. Note the antibody complexing with the flagellum (× 250).



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Urinary cast after immunoperoxidase staining. Note the absence of dark brown granular structures (× 250).

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Discussion

Although it is a relatively new technique, immunoperoxidase staining has become a very popular tool for demonstration of large numbers of different antigens in tissue sections. Similar applications have been described for the recognition of several microorganisms that may be difficult to identify, e.g., *Chlamydia*[2] and *Treponema pallidum*.[1] This paper has demonstrated that the technique is also applicable to cytologic preparations. The procedure is not difficult and, while more time consuming than routine staining, is a superior method for demonstration of *Trichomonas* organisms in cervical smears. This finding is certainly of epidemiologic interest and probably of clinical interest as well.

Perhaps the major clinical usefulness of this technique, however, relates to the examination of material from unusual sites. Figure 4 shows a urine cytology with a castlike structure containing several indistinct structures, some of which contain an "eye" suggestive of *Trichomonas*. This would be an extremely unlikely finding since the cast structure would indicate the presence of organisms within the kidney itself. Immunoperoxidase staining of the cast was, in fact, negative, confirming that these structures were not trichomonal organisms. A similar situation was noted in a cytology preparation from fluid obtained from a renal cyst (Figure 5A). The cells with a vague outline and grey blue cytoplasm (arrows) were noted by the screening cytotechnologists to bear some resemblance to *Trichomonas*. Immunoperoxidase staining of the same preparation unequivocally established that these structures were indeed *Trichomonas* (Figure 5B)—again, an unlikely diagnosis, but on in which this technique provided the final proof.

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Immunoperoxidase staining represents a relatively easy and rapid approach to the unequivocal diagnosis of *Trichomonas* infestation, regardless of the loca tion or state of preservation of the organisms or previous staining of the preparation This method would seem to have application not only for the individual case but also for the more accurate definition of the epidemiology of this very common pathogen

Acknowledgements

We are indebted to Richard J Davis M D for obtaining the cervical smears used in the study and to Linda V Kelly C T (ASCP) for the preparation of the urine slides

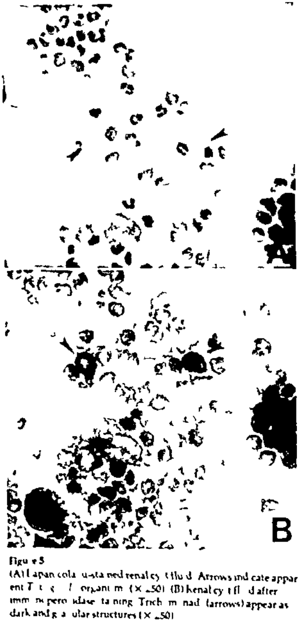

Figure 5
(A) Papanicolaou-stained renal cyst fluid Arrows indicate apparent *T vaginalis* organisms (× 250) (B) Renal cyst fluid after immunoperoxidase staining Trichomonads (arrows) appear as dark and granular structures (× 250)

References

1 Beckett JH Bigbee JW Immunoperoxidase localization of *Treponema pallidum* Arch Pathol Lab Med 103 135 138 1979

2 Bennett BD Bailey JM Gardner WA Immunocytochemical identification of trichomonads Arch Pathol Lab Med 104 247 249 1980

3 Memik F Trichomonads in pleural effusion JAMA 204 211 212 1968

4 Migaki G Benirschke K McKee AE Casey HW Trichomonal granuloma of the pelvic cavity in a rhesus monkey Vet Path 15 679-681 1978

5 Naib Z Exfoliative Cytopathology Second edition Boston Little Brown 1976 pp 90-91

6 Walton BC Bacharach T Occurrence of trichomonads in the respiratory tract Report of three cases J Parasitol 49 35 38 1963

7 Woodland RM El Sheikh H Darougar S Squires S Sensitivity of immunoperoxidase and immunofluorescence staining for detecting *Chlamydia* in conjunctival scrapings and in cell culture J Clin Pathol 31 1073 1077 1978

The Value of Ethanol as a Fixative in Urinary Cytology

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Although 50% ethanol is widely used as a fixative in urinary cytology the value of this procedure has received little systematic analysis The purpose of this study was to examine the preservative qualities of 50% ethanol when added to freshly collected urine for both immediate and delayed processing The results indicate that (1) degenerative changes occurring in vivo are common among urothelial cells and probably represent the single most important factor in cell preservation in voided urine (2) with few exceptions fresh unfixed urine yields the greatest number of well preserved cells and (3) reconstitution in either saline or 25% ethanol-saline and 24 hour storage result in increased cellular degeneration For Millipore filter preparations the addition of 50% ethanol has no apparent advantage even when processing must be delayed for several hours

Rapid fixation is important in the preparation of most cytologic material Many specimens e g cell smears from the uterine cervix can be well preserved by immediate immersion in 95% ethanol For samples composed of exfoliated cells like sputum preservation in 50% ethanol has been of value[4] Fixation of body cavity fluids and urine remains controversial largely because studies specifically designed to determine the value of this procedure have not appeared in the literature Many advocate the use of 50% ethanol i e , a 25% ethanol fluid mixture[2] while others prefer to process the fresh specimen[1,3,7] It was the purpose of this study to evaluate the usefulness of 50% ethanol as a fixative when added to freshly voided urine and to determine the efficacy of a 25% ethanol-saline solution for the preservation of urothelial cells in cold storage

Materials and Methods

Specimens were obtained from patients being evaluated for urologic disorders at the University of Tennessee Center for the Health Sciences during the period January to June 1979 Samples were refrigerated at 4 C immediately after collection and were transported to the cytology laboratory so that processing could begin within four hours of voiding All voided urine samples of sufficient volume were included The urine was thoroughly mixed divided into four aliquots of 10 ml each and initially processed in one of four manners (A) the specimen was neither fixed nor centrifuged (B) the specimen was mixed with an equal volume of 50% ethanol allowed to stand at 4 C for 30 minutes and reagitated (C) the specimen was centrifuged at 1 500 rpm for ten minutes the supernatant was discarded and the sediment was reconstituted with 0 9% saline and stored at 4 C for 24 hours or (D) the specimen was centrifuged at 1 500 rpm for ten minutes the supernatant was discarded and the sediment was reconstituted with a 25% ethanol-0 9% saline solution and stored at 4 C for 24 hours All aliquots were finally processed by standard techniques onto Millipore filters (Millipore Corporation Bedford Massachusetts 5 0μm pore size 25-mm diameter) and stained according to a modified Papanicolaou method One filter was prepared per aliquot so that a total of 236 filters were examined

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Slides were coded and evaluated according to accepted morphologic criteria of degeneration These included cytoplasmic vacuolization nuclear swelling margination of chromatin loss of chromatinic detail pyknosis and karyorrhexis The total number of urothelial cells and the number of preserved cells were recorded Mean values for cells per filter and percentage of preserved cells were calculated Statistical significance was determined by one way analysis of variance with Newman Keuls a posteriori test Only urothelial cells of intermediate and basal size were analyzed Superficial urothelial cells were excluded because they differ chemically and ultrastructurally from other urothelial cells are constantly exposed to urine and are unimportant in the cytologic interpretation of urinary specimens

Results

A total of 8 996 urothelial cells from 59 voided urine specimens were analyzed Although numerous superficial cells occurred there were relatively few intermediate and basal cells (Table I) Over half the samples contained < 25 such cells and 90% had < 100 The distribution of cells among aliquots was similar however

The percentage of preserved cells in each group paralleled that of the total number of cells (Table II) Less than 25% of the cells were well preserved in most aliquots It was unusual for an aliquot to have more than 75% well preserved elements As illustrated in Figures 1 to 3 strict criteria of cellular preservation were used Many elements with only slight changes were placed in the degenerated category

Table I *Cell [illegible] Distribution in Voided Urine Samples by Method*

	< 25	25 100	> [illegible]
A	31	[illegible]	
B	30	[illegible]	[illegible]
C	33	[illegible]	
D	31	[illegible]	[illegible]

Table II *D[illegible] of [illegible] Preserved C[illegible] Samples by Method*

	< 25%	25 75%	> 75%
A	[illegible]	[illegible]	
B	[illegible]		
C	43	[illegible]	[illegible]
D	43	[illegible]	

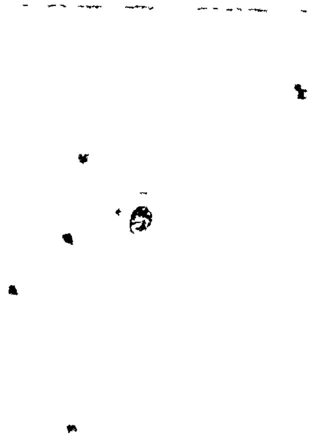

Figure 2
Well preserved urothelial cell with finely granular (dusty) chromatin surrounded by a smooth nuclear border and nonvacuolated cytoplasm (Papanicolaou stain × 630)

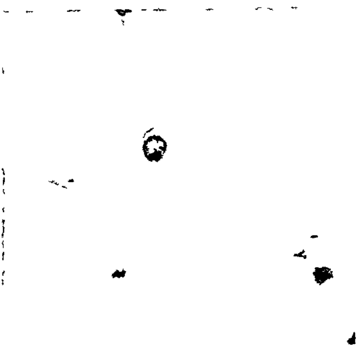

Figure 3
Partially degenerated urothelial cell Although some nuclear detail is preserved the nucleus appears swollen and the chromatin is marginated (Papanicolaou stain × 630)

Table III *Cellular Preservation in Voided Urine Samples by Method*

	Total no cells	Mean no cells per filter	Percentage of preserved cells
A	2 141	36	35 5
B	2 160	37	27 3
C	2 334	40	24 1
D	2 361	40	24 4

among individual aliquots (preservation ranged from 0 to 91%) further calculations based upon the sum of individual values were performed When this was done the average percentage of cellular preservation for each group was lowered The results of statistical analyses were the same however There was a significant difference in mean percentage preservation between groups A and C and groups A and D ($p <$ 0 05) Statistical differences were not documented between groups A and B B and C and B and D

Discussion

The cytologic evaluation of voided urine is hampered by the high percentage of poorly preserved cells in the samples Although the problem can be partially alleviated by washing the urothelium with physiologic solutions this procedure requires instrumentation with an increased risk of infection a requirement for trained personnel and increased cost Although numerous chemicals have been used ethanol is of proven value as a fixative for many cytologic specimens and has been advocated as a means of preserving urothelial cells in voided urine samples [2,4] Since it is impractical in most institutions to require patients to void into a container of alcohol the fixative has usually been added upon arrival of the specimen in the cytology laboratory A solution of 25% ethanol (equal volumes of 50% ethanol and urine) is widely used This percentage is recommended in order to lessen the shrinkage and hardening effects of more concentrated solutions while retaining the preservative qualities of ethanol

This study was designed to determine the usefulness of alcohol fixation of voided urine samples processed onto Millipore filters To minimize the effect of individual specimen variation each sample served as its own control Analysis was restricted to urothelial cells of basal and intermediate size Superficial cells were excluded because they are constantly exposed to urine not as likely to reflect its effects and more important are not useful in the cytologic interpretation of urothelial diseases

The results reaffirmed previous observations that few intermediate and basal urothelial cells occur in voided urine samples and that most of them have at least some morphologic features of degeneration[4] Degeneration varied from slight to marked and apparently occurred prior to receipt of the specimen in the laboratory

Comparison of unfixed aliquots prepared within four hours of collection (method A) to both fixed and unfixed samples stored overnight in saline (methods C and D) indicated that the greatest percentage of preserved cells was found in the former This difference was statistically significant regardless of the method of calculation of mean values

Exposure of urothelial cells to a 25% ethanol solution did not improve preservation even when the urine was replaced by an isotonic isosmotic solution such as 0 9% saline Indeed the combination of ethanol and urine apparently resulted in loss of preserved cells (method B) In filter preparations the shrinkage and hardening effects of even low concentrations of ethanol may result in sufficient cellular and nuclear changes to mimic degeneration[5]

Although not the primary purpose of this study the data can be combined with current concepts of cell membrane metabolism to gain insight into the degenerative process in urothelium as it relates to urinary cytology Exfoliated cells must be partially degenerated prior to sloughing into the urine The pH salt concentration and osmolality of urine vary markedly from those of the fluid in which intermediate and basal urothelial cells are normally bathed This should require rapid adjustments if exfoliated cells are to maintain their integrity Variations in cell preservation among individual urine samples probably reflect alterations in the composition of the urine at various times in patients with diverse urologic diseases Voided urine is immediately exposed to a lower ambient temperature and this is usually reduced even further by refrigeration The degenerative process is markedly retarded by cold storage in an isotonic isosmotic solution A low concentration of fixative such as ethanol neither augments cellular preservation nor prevents further degeneration The evidence both factual and circumstantial strongly suggests that most of the morphologic changes recognized as degenerative occur prior to voiding while the urothelial cells reside in the bladder

References

1 Barrett DL King EB Hasson PL Collection and cytopreparatory techniques for serous effusions and cerebrospinal fluids Lab Med 9 9 22 1978

2 Danos M Keebler CM Cytopreparatory techniques *In* A Manual of Cytotechnology Edited by CM Keebler JW Reagan Fifth edition Chicago American Society of Clinical Pathologists 1977 pp 264 317

3 Holmquist ND Diagnostic Cytology of the Urinary Tract *In* Monographs in Clinical Cytology Sixth volume Edited by GL Wied New York S Karger 1977 pp 69 76

4 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Philadelphia JB Lippincott 1979 pp 716 717 1165-1186

5 Linberg LG Ohlin B Specimen fixation in urinary cytology Acta Cytol 22 142 145 1978

6 Saccomanno G Saunders RP Ellis H Archer VE Wood BG Beckler PA Concentration of carcinoma or atypical cells in sputum Acta Cytol 7 305 310 1963

7 von Haam E Cytology of Transudates and Exudates *In* Monographs in Clinical Cytology Fifth volume Edited by GL Wied New York S Karger 1977 pp 107 112

Fine Needle Aspiration and Endoscopic Brush Cytology

Comparison of Direct Smears and Rinsings

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When cellular samples obtained by endoscopic brushings and by fine needle aspirations were prepared by the direct smear technique 22% were unsatisfactory due to scantiness of the cellular sample air drying of the cells or bloodiness of the specimens To improve diagnostic accuracy an additional specimen was prepared by rinsing the brush or needle in a balanced salt solution from which cells were recovered by membrane filtration The combination of direct smears and rinsings decreased our unsatisfactory rate to 2% and increased our diagnosis rate for malignant neoplasms by 24%

In view of the widespread use of endoscopic brushings and fine needle aspirations (FNA) in the cytologic diagnosis of cancer it is important for the cytopathology laboratory to be able to process small cellular samples to give a minimum number of unsatisfactory specimens and a maximum of correct diagnostic results

Until 1978 only the direct smear method was used in our laboratory in preparing specimens obtained by endoscopic brushing or FNA Unfortunately about 22% of these specimens were unsatisfactory due to scantiness of the cellular sample poor nuclear detail caused by air drying of cells before fixation or bloodiness of the specimens In an attempt to reduce this high proportion of unsatisfactory specimens we requested that clinicians rinse the endoscopic brushes and the aspiration needles after use in a balanced salt solution in addition to preparing direct smears from the brush or needle The cells were recovered from this solution by membrane filtration The purpose of this additional procedure was not only to retrieve as many cells as possible but also to improve their preservation This report records the success we have achieved by the combined use of the direct smear and rinsing techniques

Materials and Methods

During a 12 month period we examined 600 endoscopic and FNA specimens Of these 600 specimens 251 were bronchoscopic brushings 223 were brushings from the alimentary tract and 126 were fine needle aspirates from various body sites Two samples of each specimen were submitted for examination direct smears fixed with a spray fixative or 95% ethanol and brush or needle rinsings collected in a balanced salt solution (*Plasmalyte pH 7 4 Baxter* Travenol Laboratories Deerfield Illinois) The same procedures were used for bronchial alimentary and FNA specimens

The direct smears were stained by the Papanicolaou method The rinsings were prepared by a membrane filtration technique using Gelman filters (Gelman Sciences Inc Ann Arbor Michigan) and stained by a modified Papanicolaou method[2]

Preparation of Direct Brushings at Endoscopy

After the brush was withdrawn from the patient it

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was quickly smeared on a glass slide using a circular motion over an area about 1 5 cm in diameter (Figure 1) [1] Because this technique confined the specimen to a small area it prevented or reduced the tendency for air drying of the cells prior to fixation With scanty specimens when there was a danger of the cells drying before fixation a small drop of balanced salt solution was first placed on the slide to mix with the cells The material was spread as described above We recommend using a spray fixative with these specimens since the cellular suspension is somewhat thin and watery With alcohol fixation such specimens may have a greater tendency to wash off the slide

Preparation of Brush Rinsings at Endoscopy

After the direct smear was made the brush was inserted in a 50-ml centrifuge tube containing 20 to 30 ml of balanced salt solution The brush was agitated vigorously to dislodge any remaining cells caught in the bristles The rinsing specimen was then submitted to the cytopathology laboratory together with the direct smear

Preparation of Direct Smears at FNA

After the clinician removed the needle from the patient the smears were prepared by the two-slide pull apart technique The smears were then immediately spray fixed before the slightest air drying occurred When the amount of material expressed from the needle was scanty we immediately placed over the cellular material a slide on which we had already put a drop of the balanced salt solution The smears were then prepared by *the two-slide pull apart technique* and fixed with a spray fixative

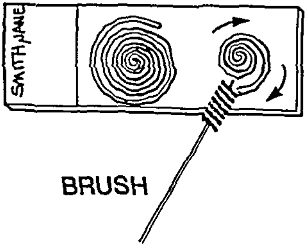

Figure 1
Brush smearing technique The cellular sample is smeared over only a small area of the slide When the amount of material on the brush is abundant a second smear may be prepared on the same slide

Preparation of Rinsings at FNA Procedures

After the direct smears were prepared the needle was rinsed by forcibly sucking balanced salt solution through the needle into the syringe and expelling it back into a centrifuge tube This procedure was repeated two or three times to ensure retrieval of all the cells in the needle and syringe

Results

Direct smears and rinsings were prepared from 600 endoscopic and FNA specimens Using the direct smear method alone 469 (78%) specimens were diagnostically satisfactory When rinses and direct smears were used together 588 (98%) specimens were diagnostically satisfactory for a 26% improvement (Table I) Thus by the addition of the rinsing method we have almost eliminated the need for repeat procedures due to unsatisfactory specimens

Tables II to IV compare the results obtained with

Table I *Combined Techniques of Smears and Rinsing Reduction in Number of Unsatisfactory Specimens*

	Unsatisfactory for diagnosis	Percent unsatisfactory	Satisfactory for diagnosis	Percent satisfactory
Smears only	103	2	469	78
Smears and rinses	1[illegible]	[illegible]	588	98

Table II *Bronchoscopic Brushing Comparison of Results from Direct Smears and Rinsings*

251 specimens (106 malignant neoplasms)		Direct smears	Rinses
Negative or unsatisfactory diagnoses			
	137	Negative	Negative
	8	Unsatisfactory	Negative
Positive diagnoses			
	84	Positive	Positive
	1	Positive	Negative
	3	Unsatisfactory	Positive
	18	Negative	Positive

Diagnosed by rinsings only 1

Table III *Endoscopic Brushings from Alimentary Tract Comparison of Results from Direct Smears and Rinsings*

223 *specimens* (22 malignant neoplasms)	Direct smears	Rinses
Negative or unsatisfactory diagnoses		
101	Negative	Negative
92	Unsatisfactory	Negative
8	Unsatisfactory	Unsatisfactory
Positive diagnosis		
13	Positive	Positive
0	Positive	Negative
7	Unsatisfactory	Positive
2	Negative	Positive

Diagnosed by rinsings only 9

Table IV *Fine Needle Aspirates from Various Sites Comparison of Results from Direct Smears and Rinsings*

126 specimens (69 malignant neoplasms)	Direct smears	Rinses
Negative or unsatisfactory diagnoses		
41	Negative	Negative
10	Unsatisfactory	Negative
6	Unsatisfactory	Unsatisfactory
Positive diagnoses		
49	Positive	Positive
2	Positive	Negative
5	Unsatisfactory	Positive
13	Negative	Positive

Diagnosed by rinsings only 18

direct smears and rinsings from the bronchial tree and alimentary tract and by FNA Of the 197 positive diagnoses of malignant neoplasms made by the combined procedures 48 (24%) were made from the rinsings only in instances where the direct smear gave unsatisfactory or negative results Figure 2 illustrates the improvement in cytologic detail obtained by use of the rinsing technique

Discussion

In our experience air drying of cells before fixation has been the major cause of unsatisfactory preparations If there is even a short delay between the collection of the cells on a brush and their being smeared on the slide or if the cells are spread over too wide an area especially in the case of brushings they are apt to dry before fixation To prevent the drying artifact with brush specimens we have used a method of spreading the cells over only a small area of the slide

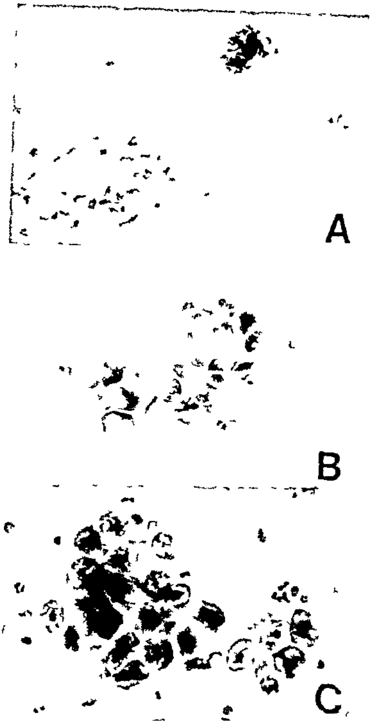

Figure 2
Gastric brushing (A) Direct smearing technique Unsatisfactory cellular preparation because the cells are scanty and air dried (× 150) (B) *Direct smear technique* Unsatisfactory cellular *preparation because the cells are air dried* (× 630) (C) *Rinsing technique with membrane filtration* Adenocarcinoma *Excellent cytologic* detail (× 630)

In addition adding a drop of balanced salt solution to the slide before the smear is made has also proven to be a useful technique for brushings or fine needle aspirates when the cellular material is scanty

In addition to these precautions we have im proved our diagnostic yield by rinsing the brushes and aspiration needles in balanced salt solution and collecting any suspended cells by the membrane filtration technique However since a suspended cellular sample in balanced salt solution may begin to autolyse within one hour after the specimen has been collected prompt handling of the rinsings is essential

Blood has also caused problems in the preparation of smear specimens Not only can blood dilute the cellular sample but air drying seemed to occur faster on these smears This problem can be overcome by working quickly with the sample or by placing a drop of balanced salt solution on the slide on which the cellular sample is to be smeared

When the amount of material collected by endoscopy or FNA was scanty the rinsing procedure ensured retrieval of virtually all cells In an attempt to improve our diagnostic results we analyzed the types of brushes used for endoscopy and the findings obtained with them The unsatisfactory results from the bronchoscopic brush specimens were less than those obtained from brushings of the alimentary tract We noticed a difference in the structure of brushes the brushes used for bronchoscopy ranged from 2 to 3 mm in length by 3 mm in diameter whereas the brushes used for alimentary samples ranged from 8 to 13 mm in length by 2 to 3 mm in diameter These different dimensions may or may not be significant We also noticed a difference in the stiffness of the nylon bristles The bristles of the brushes used for bronchoscopy were much stiffer than the soft pliable bristles of those used for the alimentary specimens This raises the question of whether the stiffer brushes would be more suitable for specimens in the alimentary tract Would they yield more cells? We are presently investigating this possibility

In the meantime we urge laboratories to supplement the direct smear technique with rinsings With the use of both techniques the percentage of unsatisfactory specimens can be reduced to a negligible figure and the percentage of malignant neoplasms diagnosed can be increased significantly

Acknowledgments

We wish to thank Dr Bernard Naylor and Ms Linda Silverman for their assistance in the preparation of this manuscript

References

1 Farrow GM Personal communication

2 Kin SR Smith MJ Diagnostic Cytology by Membrane Filter Application Bulletin 100 Ann Arbor Michigan Gelman Sciences 19 8

ference. A strong correlation of ferruginous bodies and restrictive lung disease is seen with the spontaneous specimens, but the aerosol-induced specimens show little correlation regardless of their higher levels of ferruginous body detection.

Comparison of all aerosol-induced and spontaneous sputum specimens also showed significant differences. Therefore, a second study was performed comparing 807 matched pairs of induced and spontaneous specimens from 112 individuals who had *one* or more specimens showing moderate to severe atypia or squamous carcinoma (Table I). Statistical methods used in the analysis included the paired sample t test, the Wilcoxon matched pairs signed-rank test and χ^2 tests of significance.

Results

Factors evaluated in the comparisons were: (1) the percentage of unsatisfactory specimens (defined as the absence of alveolar macrophages) as a measure of specimen quality, (2) the detection of clinically significant abnormal cytology (defined as moderate to severe atypia or carcinoma), and (3) the detection of ferruginous bodies. In reviewing the data resulting from the comparison, it was found that the laboratory cytopreparation methods and the smoking behavior of the individuals influenced the results; therefore, these factors were also considered in the analysis.

Specimen Quality

The percentage of unsatisfactory specimens, as shown in Table II, was found to be less for the aerosol-induced sputa independent of the laboratory preparation method. With the pick and smear method the qualitative difference between the two types of specimens was statistically significant. When the Saccomanno technique was used, however, the difference was not statistically significant.

Cigarette smoking did have an effect on the differences in specimen quality (Table III). The yield of satisfactory specimens was significantly increased by aerosol induction only for those individuals who *never smoked. In both smokers and exsmokers there* was no statistically significant difference between the two specimen types.

Detection of Atypical Cells

In the detection of atypical cells, 74% of the Saccomanno prepared aerosol-induced sputa yielded no atypia as compared to 66% for the spontaneous *sputa. In contrast, with the same technique, 4.6% of* spontaneous specimens showed moderate atypia, severe atypia or cancer as compared to 2.1% of the aerosol-induced specimens. However, since the finding of moderate to severe atypia or cancer prompted *the collection of three to six additional sputum speci*mens, these percentages could be misleading. For this reason, it was necessary to compare the matched

Table I Sp[illegible] (SS) [illegible] Aerosol-[illegible] (AS) Specimen

	M[illegible] [illegible]reparation		
Factor considered	Pick and smea[illegible] (304 p[illegible]	Saccomanno technique (501 pairs)	[illegible]tal (807 pairs)
C[illegible] atyp[illegible] (m[illegible] N[illegible]		[illegible] better than [illegible]ed (p=0.0[illegible])	[illegible] better than [illegible] (p=0.03)
Higher score in SS		125	1[illegible]
SS & AS same score		25[illegible]	40
Higher score in AS		0[illegible]	156
Total pairs			4[illegible]
F[illegible] (FB) [illegible] Induc[illegible] Spontan[illegible]		[illegible]ter	[illegible]ter than [illegible]=0.0[illegible]

pairs of sputum specimen types obtained from the same individuals It was then found that there was a significant difference (see Table I) with a higher degree of atypia found more frequently in the spontaneous specimens than in the aerosol induced ones

When the two methods of specimen cytopreparation were compared as shown in Table IV it was noted that only with the Saccomanno technique was there a significantly increased yield of atypical cells in the spontaneous specimens There was no significant difference between the aerosol induced and spontaneous sputa with the pick and smear method

Detection of Ferruginous Bodies

The matched pairs of aerosol induced and spontaneous specimens were also compared for their yield in the detection of ferruginous bodies (see Table I) A larger number of ferruginous bodies were more frequently detected in the aerosol induced specimens and the difference was statistically significant This is particularly interesting when one considers that the spontaneous specimens represent a three-day pooled collection whereas the aerosol specimens represent a single induction procedure

The relationship of the method of cytopreparation to the detection of ferruginous bodies is shown in Table V No significant difference was found between the two types of sputum specimens using the Saccomanno technique However there was a significant difference with the pick and smear method the aerosol induced specimens had a significantly higher yield of ferruginous bodies Also it was noted that the pick and smear technique was more effective than the Saccomanno method in ferruginous body detection for both types of sputum specimens

As with specimen quality smoking was found to influence the detection of ferruginous bodies in the two specimen types The aerosol induced specimens from smokers showed a slightly but not statistically

Table II Tec[illegible] (S[illegible] Q[illegible]lty

	Percent unsatisfactory specimen		
Specimen type	Pick and smear technique	Saccomanno technique	Total
Spontaneous	68/[illegible]000 (13.3%)	161/3 907 (4.1%)	4[illegible]9/5 9[illegible]6 (7.2%)
Induced	177/1 867 (9.4%)	139/3 591 (3.8%)	316/5 458 (5.8%)

Technique p < 0.0001 (both specimen types)
Specimen type p < 0.005 (by pick and smear technique)

T[illegible] III S[illegible] Q[illegible]

	Percent unsatisfactory specimen			
Specimen type	Never smoked	Ex-smoker	Current smoker	Total
Spontaneous	1[illegible]/[illegible]4[illegible] (14.[illegible])	15[illegible]/1 015 (8.[illegible])	1[illegible]/7[illegible] (1[illegible])	4[illegible]/5 [illegible]5 (7.[illegible])
Induced	[illegible]/[illegible] (10.[illegible])	1[illegible]/1 65[illegible] (7.[illegible])	[illegible]/[illegible]00 (3.8)	31[illegible]/5 [illegible]01 ([illegible].1)
S[illegible]	p = 0.00	p = 0.55	p = 0.10	

T[illegible] IV Te[illegible] A[illegible] C[illegible] (A[illegible]) [illegible]

	Specimens with abnormal cytology		
Specimen type	Pick and smear technique	Saccomanno technique	Total
Spontaneous	3[illegible]/1 6[illegible] ([illegible])	163/3 3[illegible] (4.9%)	19[illegible]/5 0[illegible] (3.9%)
Induced	[illegible]/1 [illegible]0 (1.9%)	[illegible]/[illegible] ([illegible].0%)	[illegible]/4 [illegible]16 (1.9)

Technique p < 0.001 [illegible] spontaneous specimens
Specimen type p < 0.001 [illegible] Saccomanno technique

Tabl[illegible] V Tec[illegible] (F[illegible] B[illegible] (A[illegible]) [illegible]

	Specimens with ferruginous bodies		
Specimen type	Pick and smear technique	Saccomanno technique	Total
Spontaneous	3[illegible]/1 6[illegible] (1[illegible].3%)	4[illegible]/3 3[illegible] (1.[illegible]9)	[illegible]41/5 0[illegible] (14.[illegible])
Induced	3[illegible]/1 [illegible]60 ([illegible]3.5)	350/[illegible] (11.8%)	[illegible]/4 516 (15.9%)

Technique p < 0.0001 (highly significant)
Specimen type p = 0.13 (not significant) by Saccomanno technique p = 0 (not significant) by pick and smear technique p = 0.000[illegible] (highly significant)

ference A strong correlation of ferruginous bodies and restrictive lung disease is seen with the spontaneous specimens but the aerosol induced specimens show little correlation regardless of their higher levels of ferruginous body detection

Comparison of all aerosol induced and spontaneous sputum specimens also showed significant differences Therefore a second study was performed comparing 807 matched pairs of induced and spontaneous specimens from 112 individuals who had one or more specimens showing moderate to severe atypia or squamous carcinoma (Table I) Statistical methods used in the analysis included the paired sample t test the Wilcoxon matched pairs signed-rank test and χ^2 tests of significance

Results

Factors evaluated in the comparisons were (1) the percentage of unsatisfactory specimens (defined as the absence of alveolar macrophages) as a measure of specimen quality (2) the detection of clinically significant abnormal cytology (defined as moderate to severe atypia or carcinoma) and (3) the detection of ferruginous bodies In reviewing the data resulting from the comparison it was found that the laboratory cytopreparation methods and the smoking behavior of the individuals influenced the results therefore these factors were also considered in the analysis

Specimen Quality

The percentage of unsatisfactory specimens as shown in Table II was found to be less for the aerosol induced sputa independent of the laboratory preparation method With the pick and smear method the qualitative difference between the two types of specimens was statistically significant When the Saccomanno technique was used however the difference was not statistically significant

Cigarette smoking did have an effect on the differences in specimen quality (Table III) The yield of satisfactory specimens was significantly increased by aerosol induction only for those individuals who never smoked In both smokers and exsmokers there was no statistically significant difference between the two specimen types

Detection of Atypical Cells

In the detection of atypical cells 74% of the Saccomanno prepared aerosol induced sputa yielded no atypia as compared to 66% for the spontaneous sputa In contrast with the same technique 4 6% of spontaneous specimens showed moderate atypia severe atypia or cancer as compared to 2 1% of the aerosol induced specimens However since the finding of moderate to severe atypia or cancer prompted the collection of three to six additional sputum specimens these percentages could be misleading For this reason it was necessary to compare the matched

Table I *Spontaneous (SS) versus Aerosol Induced (AS) Specimens*

	Method of preparation		
Factor considered	Pick and smear technique (304 pairs)	Saccomanno technique (501 pairs)	Total (807 pairs)
Cellular atypia [illegible]			
	No significant difference (p = 0 55)	Spontaneous better than induced (p = 0 02)	Spontaneous better than induced (p = 0 03)
Higher score in SS	61	125	187
SS & AS same score	147	255	402
Higher score in AS	59	97	156
Total pairs	267	477	745
Ferruginous bodies (FB) [illegible]			
	Induced better than spontaneous (p < 0 001)	No significant difference (p = 0 45)	Induced better than spontaneous (p = 0 0[illegible])
More FB in SS	35	71	106
SS & AS same	[illegible]	[illegible]	14
More FBs in AS	63	55	123
Total pairs	10[illegible]	136	243

Two pairs were not matched for preparation method 62 pairs had one or both specimens unsatisfactory and 564 pairs had no ferruginous bodies present pairs of sputum specimen types obtained from the same individuals It was then found that there was a significant difference (see Table I) with a higher degree of atypia found more frequently in the spontaneous specimens than in the aerosol induced ones

When the two methods of specimen cytopreparation were compared as shown in Table IV it was noted that only with the Saccomanno technique was there a significantly increased yield of atypical cells in the spontaneous specimens There was no significant difference between the aerosol induced and spontaneous sputa with the pick and smear method

Table II Technique and Specimen Quality

Specimen type	Percent unsatisfactory specimens Pick and smear technique	 Saccomanno technique	Total
Spontaneous	[illegible]8/[illegible] 009 (13 3[illegible])	161/3 967 (4 1[illegible])	4[illegible]9/5 9[illegible]6 (7 [illegible])
Induced	17[illegible]/1 867 (9 4%)	139/3 591 (3 8[illegible])	316/5 458 (5 8%)

Technique p < 0 0001 (both specimen types)
Specimen type p < 0 005 (by pick and smear technique)

Table III Smoking and Sputum Quality

Specimen type	Percent unsatisfactory specimens Never smoked	 Ex-smokers	 Current smokers	Total
Spontaneous	144/9[illegible]1 (14 7 [illegible])	156/1 915 (8 1 [illegible])	1[illegible]9/[illegible] (4 [illegible])	4[illegible]9/5 [illegible]5 (7 6 [illegible])
Induced	95/944 (10 1 [illegible])	1[illegible]6/1 [illegible]5 ([illegible])	98/[illegible]600 (3 8 [illegible])	310/5 [illegible]01 (6 1 [illegible])
Significance	p = 0 00	p = 0 55	p = 0 10	

Table IV Technique and Abnormal Cytology (Nonsmokers Only)

Specimen type	Specimens with abnormal cytology Pick and smear technique	 Saccomanno technique	Total
Spontaneous	15/1 [illegible]6 ([illegible] 0%)	163/3 336 (4 9%)	10[illegible]/5 0[illegible] (3 9 [illegible])
Induced	[illegible]9/1 560 (1 0 [illegible])	58/[illegible]056 ([illegible] 0 [illegible])	[illegible]8/4 516 (1 9 [illegible])

Technique p < 0 001 (for spontaneous specimens)
Specimen type p < 0 001 (by Saccomanno technique)

Table V Technique and Ferruginous Bodies (Asbestos Workers Only)

Specimen type	Specimens with ferruginous bodies Pick and smear technique	 Saccomanno technique	Total
Spontaneous	300/1 6[illegible] (19 3[illegible])	43/3 336 (1 [illegible]9[illegible])	[illegible]41/5 0[illegible] (14 [illegible])
Induced	36[illegible]/1 560 (23 5[illegible])	350/[illegible]056 (11 8[illegible])	[illegible]17/4 516 (15 9 [illegible])

Technique p < 0 0001 (highly significant)
Specimen type p = 0 13 (not significant) by Saccomanno technique p = 0
(not significant) by pick and smear technique p = 0 0003 (highly significant)

Detection of Ferruginous Bodies

The matched pairs of aerosol induced and spontaneous specimens were also compared for their yield i the detection of ferruginous bodies (see Table I) A larger number of ferruginous bodies were more frequently detected in the aerosol induced specimens and the difference was statistically significant This i particularly interesting when one considers that th spontaneous specimens represent a three-day poole collection whereas the aerosol specimens represent single induction procedure

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As with specimen quality smoking was found t influence the detection of ferruginous bodies in the two specimen types The aerosol induced specimen from smokers showed a slightly but not statistically

Letters to the Editors

Identification of Papillary Hidradenoma of the Vulva by Imprint Cytology

To the Editors

Cytologic diagnosis is currently used for a variety of pathologic states in many sites Since many lesions are accessible to biopsy however they are seldom described from a cytologic point of view This is true for many vulvar lesions Koss in the 1968 edition of his textbook[3] stated that cytological assessment of lesions such as papillary hidradenoma could theoretically be possible but there are no reports at hand Dupre Froment[1] claimed in 1974 that this cytologic diagnosis had been made but to our knowledge there were no photomicrographs of the cells in the literature at that time Schramm[4] however recently published the report of a case diagnosed by colposcopy with cytology in which cells from vulvar smears obtained by both cotton swab and wooden spatula were depicted

We recently had the opportunity of examining

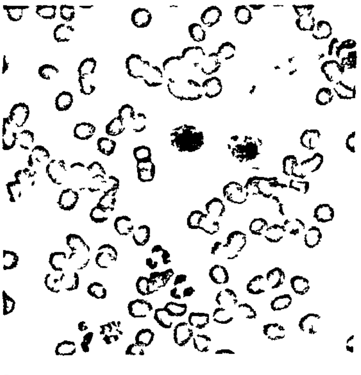

Figure 1
Touch imprint smear shows glandular cells of uneven size with fine and evenly distributed chromatin network (Papanicolaou stain × 400)

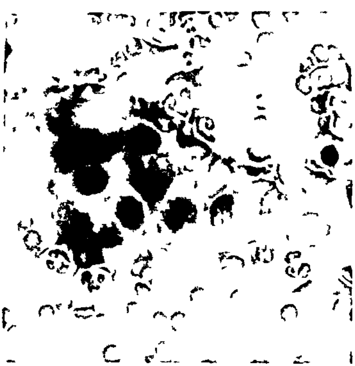

Figure 2
Touch imprint smear shows clump of glandular cells (Papanicolaou stain × 400)

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Acta Cytologica

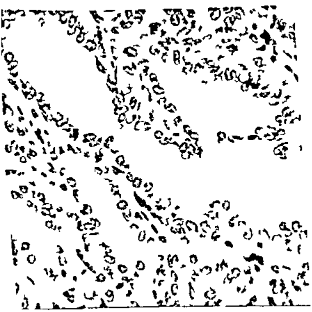

Figure 3
Excised tumor shows bistratified ducts resembling sweat glands (hemalum erythrosine saffran × 250)

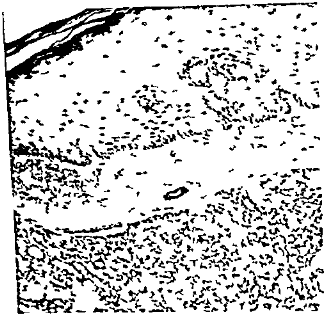

Figure 4
Excised tumor shows bistratified ducts under normal epidermis (hemalum erythrosine saffran × 100)

smears obtained by touch imprint of a bleeding vulvar lesion in an otherwise normal 45 year-old woman The smears contained blood leukocytes squamous cells and small cells either isolated or in small clusters These assumed a glandular configuration with slight anisonucleosis The nuclei had a fine and evenly distributed chromatin pattern (Figures 1 and 2) In some cells the cytoplasm was abundant and somewhat granular

It was suggested that the lesion was benign and could be a papillary hidradenoma The tumor was excised and histologic examination of the specimen confirmed the cytologic evaluation (Figures 3 and 4) The clue to the cytologic diagnosis was the presence of benign looking glandular cells in the smear obtained by touch imprint of the vulvar lesion

The differential diagnosis must include extramammary Paget s disease and a rare adenocarcinoma originating either in the sweat glands of the labia or in Bartholin s gland [2] Paget s cells are readily identified by their isolated arrangement and rounded appearance Cells from an adenocarcinoma would have obvious malignant features

Jean Hustin M D
Michael Donnay M D
Jacques Hamels M D
From the Institut de Morphologie Pathologique B 6270 Loverval Belgium

References

1 Dupre Froment J Cytologie Gynecologique Paris Flammarion 1974 p 41

Frable WJ Goplerud DR Adenoid cystic carcinoma of Bartholin s gland Diagnosis by aspiration biopsy Acta Cytol 19 15 153 1975

3 Koss LG Diagnostic Cytology and Its Histopathologic Bases Second edition Philadelphia JB Lippincott 1968 p 4

4 Schramm G Diagnosis of a papillary hidradenoma of the vulva by simultaneous cytology and colposcopy Acta Cytol 23 57 60 1979

Thin Needle Aspiration Biopsy

To the Editors

I much appreciated the wise editorial of Dr Koss[1] in the January-February 1980 *Acta Cytologica* and as a typical professional hybrid between a clinician and a cytologist I am willing to certify that he has correctly perceived the situation in Sweden in the 40s and 50s Advanced clinical diagnosis and surgery were then performed in hospitals far away from the *nearest pathology lab As an internist with an old* and passionate love of cells I soon found that I could give my colleagues (and also myself) much appreciated *immediate* help with a thin needle and a microscope The cytologic spectrum was incredibly wide (who nowadays is engaged e g in the puncture cytology of glioblastoma multiforme?) and the conclusions had to be guarded I soon learned that conclusions e g from a thyroid aspirate had to be formulated differently for colleagues than if the patient was in my personal care

This is now history *but it should not be forgotten* that aspiration cytology is a multifocal process that has always been developed by clinicians to meet clinical needs Pathologists to whom I sent my aspirates for consultation were rarely enthusiastic and sometimes hardly even polite

This brings me back to the question of professional hybrids In medicine are they wanted or unwanted? *They are usually looked upon with suspicion by all* purebred friends (in Swedish *hybrid* is in fact synonymous with *bastard* although the word cannot be used as an invective)

Personally I feel strongly (of course) for such hybrids They may help to solve one of the main problems of medicine in our time its disintegration even decay into technical specialties In this case they may even bridge the dangerously widening gulf between clinical and laboratory medicine that today affects the whole realm of medicine

Now it happens that bedside clinical work and clinical cytology are closely related in their attitudes to disease which they both conceive as coherent pictures and not as *figures and formulas In addition* the technology of the cytologist microscopy should not be beyond the reach of clinicians Thus this is a field where hybridization is natural either the pathologist with immediate contact with patients or the clinician who *is familiar with the use of microscopes* and the appearance of cells

Traditions may be different in different countries *but I believe that* cytology has here a special responsibility to show that clinical medicine and laboratory medicine can still be held firmly together—by hybridization Spontaneous hybrids are rare in nature but they do exist sometimes it may be wise to increase their numbers with art

Nils Soderstrom M D F I A C
Emeritus Professor in General Medicine Lund
Sweden

Reference

1 Koss LG Thin needle aspiration biopsy Acta Cytol 24 1 3 1980

To the Editors

The importance of thin needle aspiration biopsies of *human tissues is documented by the fact that at the* recent Seventh International Congress of Cytology in Munich a panel as well as 60 of the 248 papers scheduled for presentation dealt with this technique The approximately 50 000 aspirations cited seem to represent a considerable amount of experience compared with the total of all diagnostic procedures however that figure is less impressive This again raises questions addressed by Dr Koss[1] in his editorial in the January-February 1980 *Acta Cytologica* (1) why are more thin needle aspiration biopsies per*formed in Europe than in the United States and* (2) could insufficiencies of the service of European pathologists be one of the reasons?

For many years the performance of thin needle aspiration biopsies was centered at places such as the Karolinska Sjukhuset in Stockholm Sweden and the medical facilities in Ljubljana Yugoslavia where weaknesses in the pathology services should be excluded as a cause In fact its recent spread has been due to the adoption of the technique by radiologists *a group of clinicians not in direct contact with the pathologists A second topic is the so-called profes*sional hybrid clinical cytologist whom Dr Koss mentions We know from plant breeding that hybrids *are especially vigorous but we also know that they* do not propagate Many of us know of clinical cytol ogists who specialized in the diagnosis of one organ or another but the specialty collapsed at their hospitals the moment they departed I therefore believe that we must be concerned with breeding the pure lines the trained clinician capable of obtaining adequate diagnostic material and the trained cytologist capable of judging the parameters pertaining to the different organs

Peter Pfitzer M D F I A C
From the Department of Cytopathology Pathology Institutes University of Dusseldorf Dusseldorf
West Germany

Reference

1 Koss LG Thin needle aspiration biopsy Acta Cytol 24 1 3 1980

To the Editors

I wish to make some brief comments on the editorial by Dr Koss[5] on the subject of thin needle aspiration biopsies

If Koss is correct that I do have a great deal of interest in and knowledge about cells human cells in particular it is because of what I have been stimulated to learn by and from him Indeed Koss s paper[4] on the history of exfoliative cytology was a major factor in the writing of my article [1] I have frequently wondered why such innovations as exfoliative cytology took so long to be accepted by the medical community I am astonished that even today more than 50 years after Babes and Papanicolaou there are women in America who have not had Papanicolaou smears As Koss points out even the fiery temper of Virchow could not expedite the process of acceptance of histologic (cytologic) diagnosis in the United States in less than 30 years Readings in the history of medicine show that there are repeated instances of methods requiring decades before widespread use this was the case with the electrocardiogram and even the stethoscope Since I have used needle aspirations for obtaining cells for experimental work it was only natural that I should examine needle aspiration biopsy as one more example of the process of innovation

Koss s stimulating editorial[5] reiterated several reasons I cited in my article for the slow adoption of thin needle (0 5 to 0 9 mm OD) aspiration biopsies adequately trained personnel are not available and the belief that such biopsies represent second-class methods in comparison with current American practice I would venture another reason as yet the technique has not found the brilliant and articulate advocates in the United States that exfoliative cytology has had these past 30 years The few practitioners of the method have published relatively little until recent years and with the exception of Frable [2] who shared his experiences promptly and Koss who recognized many years ago the importance of including a chapter on aspiration cytology in his book [3] the American literature is rather sparse when compared to the European

One would hope that pathologists and cytopathologists will heed the advice and set about to standardize the training of needle aspiration cytologists through training grants demonstration projects and field trials whether funded by federal or private sources so that some day this Scandinavian curiosity will flourish with the same vitality in the United States and the rest of the world

Cecil H Fox Ph D
From the Department of Cellular Pathology Armed Forces Institute of Pathology Washington D C
U S A

References

1 F CH Innovation in medical diagnosis The Scandinavian curiosity Lancet 1 1387 1388 1979

2 Frable WJ Thin-needle aspiration biopsy A personal experience with 469 cases Am J Clin Pathol 65 168 18 1976

3 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Philadelphia JB Lippincott 1979

4 Koss LG A quarter century of cytology Acta Cytol 21 639 64 1977

5 Koss LG Thin needle aspiration biopsy Acta Cytol 24 1 3 1980

However it is as a teacher and a commanding personality that Dr Wachtel will be remembered best by her many students She ran one of the five National Schools of Cytology in the United Kingdom and Hammersmith became the meeting place for experts and trainees in cytology from all over the world The clarity of her teaching and the dry wit that accompanied it left a lasting impression on all who knew her She never forgot her students and devoted much time to solving their problems personal and professional

Dr Wachtel s interest in her stamp collection and garden demonstrated another side of her character The diversity of her knowledge was displayed by her success in completing *Daily Telegraph* crosswords for which she won no fewer than 19 prizes As a raconteur she was supreme and on social occasions she was an outstanding figure

Dr Wachtel is survived by her husband Harry Zentler-Gordon and her daughter Karen

Helena E Hughes
O A N Husain
J Elizabeth Macgregor
Arthur I Spriggs

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Announcements

Forthcoming Events Sponsored by the International Academy of Cytology

April 1-4 1981	*Third International Tutorial on Management of the Patient with Early Cervical and Intrauterine Neoplasia*	Universitats Frauenklinik Freiburg West Germany
May 9-16 1981	*19th Tutorial on Clinical Cytology*	Center for Continuing Education University of Chicago Chicago Illinois U S A
January 16-23 1982	*20th Tutorial on Clinical Cytology*	Sheraton Universal Hotel North Hollywood California U S A
January 24-26 1982	*1982 International Conference on High Resolution Cell Image Analysis*	Sheraton Universal Hotel North Hollywood California U S A
May 8 15 1982	*21st Tutorial on Clinical Cytology*	Center for Continuing Education University of Chicago Chicago Illinois U S A
October 23 30 1982	*Tenth International Tutorial on Clinical Cytology*	Vienna Intercontinental Hotel Vienna Austria
June 19 23 1983	*Eighth International Congress of Cytology*	Queen Elizabeth Hotel Montreal Quebec Canada
June 24 26 1983	*Fourth International Conference on Automation of Cancer Cytology and Cell Image Analysis*	Montreal Quebec Canada
August 13 20 1983	*22nd Tutorial on Clinical Cytology*	Center for Continuing Education University of Chicago Chicago Illinois U S A

For information on the Eighth International Congress of Cytology (1983) Montreal Quebec Canada write to

Office of the Secretary-General
Eighth International Congress of Cytology
1050 Chemin Ste Foy
Quebec P Q G1S 4L8
Canada
Telephone 418/688 3640

For information on all other events write to

Committee on Continuing Education
International Academy of Cytology
5841 South Maryland Avenue—HM 449
Chicago Illinois 60637
U S A
Telephone 312/947 5343

The International Academy of Cytology, Cytotechnology Registry and Certification Committee Successful Examinees

The following cytotechnologists have taken and successfully passed the Registry Examination for Cytotechnologists and are entitled to use the abbreviations C T (I A C) following their names

Santiago de Compostela Spain—May 27 1980

	Registration Number
Jose Luis Delgado Rioja 53-5C Leganes Madrid Spain	2019
Ma Carmen Catalan Equillor Avda de Galicia 6 2 dcha Pamplona Navarra Spain	2020
Ana Maria Funes Artiaga Frambueso No 10-70-3 Cornelia de Llobregat Barcelona Spain	2021
Roser Brossa Oliveras Bellavista No 2 Atico lo Derecha L'Hospitalet de Llobregat Barcelona Spain	2022
Ana M Carreras Marcer Valencia 4-6 20 20 Barcelona 15 Spain	2023
Maria Jesus Escudero Bueno Estudios No 4 2A Valladolid Spain	2024
Yolanda Murillo Estafeta No 39-4 *Pamplona Spain*	*2025*
Ma Paz de Villalonga Valcarcel Reina Victoria 18 Barcelona 21 Spain	2026
Mercedes Parcerisa Milanesado 3 bis 40-1 Barcelona 17 Spain	2027
Carmen Jaime-Nunez Retorno Justo Perez Medina 9 Unidad CTM Atazacoalco D F Mexico 14 Mexico	2028

ACTA CYTOLOGICA
THE JOURNAL OF CLINICAL CYTOLOGY

Editorial

On the History of Cytology

Leopold G Koss M D F I A C

An unexpected and most generous benefit of the recent Seventh International Congress of Cytology in Munich was a book *History of Clinical Cytology* that was distributed to the participants [1] The book modestly subtitled *A Selection of Documents* was written and compiled by Drs Heinz Grunze and Arthur Spriggs The two authors share an interest in cytology and its history but have very divergent backgrounds Dr Grunze is an internist and Professor of Medicine at the Medical School in Aachen Germany whereas Dr Spriggs is a pathologist and Head of the Laboratory of Clinical Cytology at the Churchill Hospital in Oxford England This association of a clinician and a pathologist is most fortuitous and is happily reflected in the pages of the substantial and lavishly illustrated book

How does one recapitulate the history of science? Is it merely a review and a selection of documents published or unpublished? Or is it a history of human thought as it fertilized and cross fertilized the receptive minds of people who were fortunate enough to be in the right place at the right time with the right person? What is more important in the progress of science a published paper or a conversation or discussion that leads to the sudden sometimes blinding recognition of the direction to be taken or discovery to be made? It is my belief that much that is happening in science in general that is worthwhile as opposed to merely minor technical improvements is the result of the process of exchange of thought

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From the Department of Pathology Montefiore Hospital and Medical Center Albert Einstein College of Medicine Bronx New York

Dr Koss is Professor and Chairman

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Recent Advances in Endometrial Neoplasia

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Dr Koss

Within recent years there has been a major increase in the rate of endometrial carcinoma in the United States perhaps because of the administration of exogenous estrogens At the same time there has been a resurgence in basic research pertaining to biologic mechanisms of this disease Naturally the means of diagnosis of a frequent and potentially fatal disorder must be redefined For these reasons this panel will examine first the epidemiologic aspects of endometrial cancer This will be followed by presentations on the subject of biochemical mechanisms of the disease During the second part of this symposium we will examine the diagnostic aspects of endometrial cancer by histologic and cytologic techniques and review some of the most recent observations on endometrial morphology and function by means of new techniques Finally I shall briefly summarize recent progress in the detection of endometrial cancer in asymptomatic women

Epidemiology of Endometrial Cancer (Dr Cramer)

Dr Cramer

In this presentation on the epidemiology of endometrial cancer I will describe its variation by age race and geography discuss the personal risk factors such as obesity and the use of exogenous estrogens and conclude with some ideas on the prevention of endometrial cancer as suggested by its epidemiology

There are several cautions to keep in mind in examining the incidence rates of endometrial cancer First in some registries coding may be so poor that it is not clear whether the cancer originated in the body or the cervix of the uterus These uterus unspecified cases have made interpretation of time trends for endometrial cancer difficult Even when a cancer is assigned to the corpus uteri we cannot be entirely certain that it is an endometrial cancer Undoubtedly a few sarcomas of the corpus will be included under this category unless the histology is also available Another problem relating to histology is that it may be difficult to distinguish a hyperplasia from an early cancer The final problem is that in areas where the prevalence of hysterectomy is high the rates generally calculated for endometrial cancer underestimate the risk to women who have intact uteri [12]

Figure 1 shows the age-specific incidence of cancer of the corpus around 1970 in some areas of the world at low and high risk for the disease The shapes of

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Presented at the 27th Annual Scientific Meeting of the American Society of Cytology San Francisco California November 2 1979

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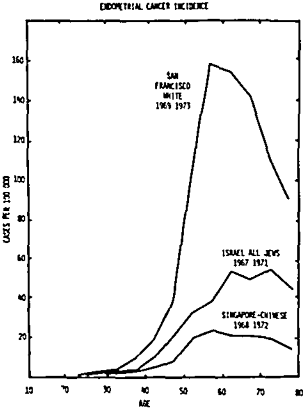

Figure 1
Age-specific incidence of endometrial cancer in low and high risk areas around 1970

these curves are basically similar they show an increase beginning in the woman s 40s a peak incidence around age 65 to 75 and a decline thereafter In Singapore the incidence rate at age 60 is about 20 cases per 100 000 in Israel the incidence rate is about 52 cases per 100 000 and in San Francisco among white females the incidence is about 156 cases per 100 000

To elaborate on the meaning of these rates an incidence of 20 cases per 100 000 at age 60 obviously means that of 100 000 60-year-old women 20 will develop the disease in a year s time But it also means that the risk that a 60-year-old native woman from Singapore will have endometrial cancer diagnosed in a year is 20 of 100 000 or 0 02% Now to obtain the risk for disease throughout the woman s lifetime one integrates the area under these curves to obtain the cumulative incidence or lifetime risk of the disease I have calculated these lifetime risks for cancer of the corpus from the age-specific rates recorded in the 1976 edition of *Cancer Incidence in Five Continents* [13] These risks vary from 0 2% among women in India to an incredible 4% among white females in San Francisco (Figure 2) For comparison the lifetime risk for breast cancer in U S females is about 8%

In general the incidence of endometrial cancer is lowest in the developing countries and higher in Western and industrialized ones Racially blacks and orientals have a lower incidence of endometrial cancer and Jewish women and caucasians have a higher incidence of the disease Obviously the striking feature of Figure 2 is that for all racial groups the women of San Francisco had the highest incidence of endometrial cancer In the 1970s San Francisco undoubtedly had the highest incidence of endometrial cancer in the world I will speculate on the reasons below

Thus demographic factors of obvious importance in the epidemiology of endometrial cancer include age race geography and socioeconomic status Other correlations that have been made with the geo

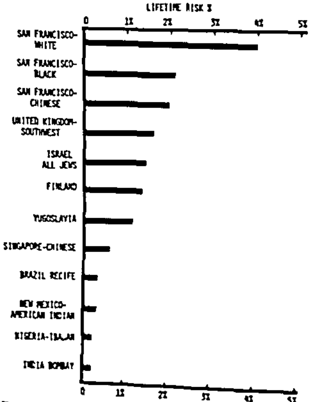

Figure 2
Cumulative incidence (lifetime risk) for endometrial cancer in low and high-risk areas around 1970

graphic differences include food-consumption patterns Armstrong and Doll correlated estimates of the per capita daily fat consumption with the incidence of endometrial cancer in various countries of the world[1] The countries that had the highest per capita fat consumption had the highest incidence of endometrial cancer Countries that had a lower per capita fat consumption and conversely higher cereal and grain consumption had the lowest incidence of endometrial cancer

Let me now move on to the personal risk factors for endometrial cancer They are often identified from epidemiologic studies of the case-control variety Exposure for example to a certain drug in women with endometrial cancer is compared with that in control women without the disease The measure of association between exposure and disease is the relative risk A relative risk of two implies that women with the exposure under investigation have twice the risk of developing the disease as women without that exposure

The following risk factors have been identified for endometrial cancer obesity diabetes hypertension liver disease ovarian diseases (such as Stein Leventhal syndrome and granulosa tumors) cancer of the breast or ovary multiparity late menopause pelvic irradiation and exogenous estrogen Obesity is a very strong risk factor for endometrial cancer In an excellent study Wynder et al compared the risk for endometrial cancer in women of various weight for height categories[15] Relative to women who were of average weights for their heights the risk ratio is less than unity for women who are underweight That means that leanness protects against endometrial cancer For women who are overweight for their height the risk increases progressively Women 10 to 20 pounds overweight had a twofold greater risk as compared to those of average weight Women who were 21 to 50 pounds overweight had a threefold greater risk And women who were more than 50 pounds overweight had a tenfold greater risk as compared to those of average we[illegible] their heights
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endometrial cancer with a number of other medical conditions such as liver disease and ovarian disease including the Stein Leventhal syndrome and granulosa tumors of the ovary[3,8,10] Endometrial cancers also have been reported in women who had cancer of other reproductive organs, specifically the breast and ovary[8]

There are numerous events in a woman s reproductive life which are important risk factors for the disease Childless women have rates of endometrial cancer twice as high as do women with one child and about three times as high as do multigravid women[4] For the woman who undergoes a late menopause there is also an increased risk For example women who undergo menopause after age 52 have about twice the risk for endometrial cancer as do women who undergo menopause at an earlier age[4] Endometrial cancer is rare among women who have had surgical castration with retention of the uterus at an early age[5]

Exposure to radiation is another well-documented risk factor for endometrial cancer In a follow up of women who received pelvic irradiation for benign or malignant diseases the rates of endometrial cancer were about eight times higher than expected[7]

The final factor I wish to discuss is the use of exogenous estrogen the use of replacement estrogens in the menopause Though controversy followed the initial reports of an association between estrogens in the menopause additional evidence has demonstrated that there is a valid and probably causal association between their use and endometrial cancer The features that suggest this association are as follows First it is a consistent and strong association Next there is a dose response Third certain time and regional correlations of drug use and disease are consistent with a causal relationship Finally the association has biologic credibility

At least seven case-control studies have shown that there is a consistent and significant association be
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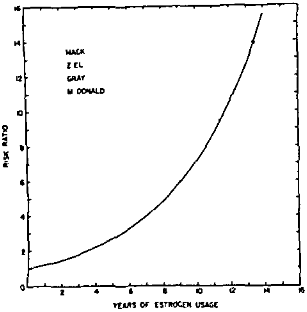

Figure 3
Dose response between endometrial cancer risk and length of estrogen use (reprinted from Cramer and Knapp with permission)

studies of endometrial cancer relevant to the years of estrogen use and the risk ratio for endometrial cancer There is an increasing risk for endometrial cancer with years of estrogen use One can fit either an exponential curve or a linear one to the data points I think the linear curve is the better fit With the linear curve risks for endometrial cancer would increase approximately 75% with each year of use With the exponential model the risk is relatively small for the first four years and then increases rapidly thereafter doubling about every four years I think we can say then that there is a clear dose response

The next point to consider in terms of estrogens and endometrial cancer is the correlation of time and regional variations in endometrial cancer incidence with variations in the drug usage I discussed above the high incidence of endometrial cancer in San Francisco and surveys have indicated that this incidence correlates with an estrogen usage that is much higher than in other areas of the United States [11] The time trend for endometrial cancer is also of value Weiss et al described a sharp increase in the incidence rates of endometrial cancer in this country occurring in the 1970s [11] This increase in incidence has been preceded by greater usage of exogenous estrogen as documented by Department of Commerce records However more recent reports indicate a sharp decline in estrogen use it has decreased by 20% alone from 1975 to 1977 One would reasonably ask will a corresponding decline in endometrial cancer incidence now be seen? Some early reports based on hospital discharge records indicate that this may be the case * If this temporal association between estrogen usage and endometrial cancer incidence is confirmed it would provide very compelling evidence for a causal association between exogenous estrogens and endometrial cancer

The final point that may be brought to bear on the estrogen–endometrial cancer relationship is biologic credibility There is a single theory that unifies most of the diverse epidemiologic associations that we have discussed namely that risk is mediated through estrogen metabolism It appears that any factor which leads to an excess of estrogens relative to progesterone may increase the risk for endometrial cancer (Figure 4) This excess of estrogen may occur through increased production from the ovary as in stromal hyperplasia tumors or anovulation or from the adrenal gland again from hyperplasia or tumors It may occur in obesity because of increased peripheral conversion of estrogen precursors or it may occur because of decreased degradation as occurs in impaired liver function Finally it may occur from exogenous intake That estrogens may be related to endometrial cancer is not a new theory but what is new is that our unfortunate experience with exogenous estrogens has provided as it were a natural experiment to test this hypothesis The strong association that has been found between estrogens and endometrial cancer thus lends strong support to this hypothesis

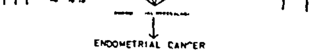

Figure 4
Model of endometrial cancer development through states which produce an excess of estrogen relative to progesterone

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Obesity may account at least in part for two other risk factors that have been identified for endometrial cancer adult-onset diabetes and hypertension both of which have been found to increase the risk for the disease about twofold[7] Case reports link endometrial cancer with a number of other medical conditions such as liver disease and ovarian disease incuding the Stein Leventhal syndrome and granulosa tumors of the ovary[3,8,10] Endometrial cancers also have been reported in women who had cancer of other reproductive organs specifically the breast and ovary[9]

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Exposure to radiation is another well-documented risk factor for endometrial cancer In a follow up of women who received pelvic irradiation for benign or malignant diseases the rates of endometrial cancer were about eight times higher than expected[7]

The final factor I wish to discuss is the use of exogenous estrogen the use of replacement estrogens in the menopause Though controversy followed the initial reports of an association between estrogens in the menopause additional evidence has demonstrated that there is a valid and probably causal association between their use and endometrial cancer The features that suggest this association are as follows First it is a consistent and strong association Next there is a dose response Third certain time and regional correlations of drug use and disease are consistent with a causal relationship Finally the association has biologic credibility

At least seven case-control studies have shown that there is a consistent and significant association between estrogen exposure in the menopause and endometrial cancer[2] The only major study not finding this association has serious methodologic flaws The association is a strong one and averages about fivefold for the estrogen user that is the user of estrogen has about five times the risk for endometrial cancer as does the nonuser However the risk does vary with the dose and length of usage For example users of 1 mg or more of conjugated estrogens had twice the risk of users of less than 1 mg

Figure 3 shows the variation by length of usage I have combined data from the initial case-control

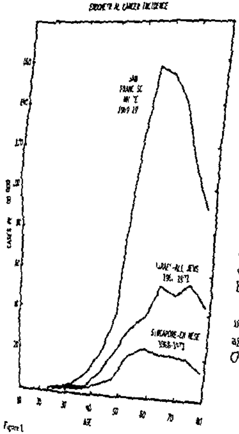


Figure 1
Age-specific incidence of endometrial cancer in low- and high-risk areas [illegible]

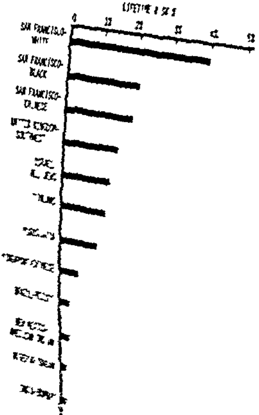
1976 edition of *Cancer Incidence in Five Continents*.[11] These risks vary from 0.2% among women in India to an incredible 4% among white females in San Francisco (Figure 2). For comparison, the lifetime risk for breast cancer in U.S. females is about 8%.

In general, the incidence of endometrial cancer is lowest in the developing countries and higher in Western and industrialized ones. Racially, blacks and orientals have a lower incidence of endometrial cancer, and Jewish women and caucasians have a higher incidence of the disease. Obviously, the striking feature of Figure 2 is that for all racial groups the women of San Francisco had the highest incidence of endometrial cancer. In the 1970s San Francisco undoubtedly had the highest incidence of endometrial cancer in the world. I will speculate on the reasons below.

Thus, demographic factors of obvious importance in the epidemiology of endometrial cancer include age, race, geography, and socioeconomic status. Other correlations that have been made with the pre

these curves are basically similar; they show an increase beginning in the women's 40s, a peak incidence around the 60s to 70s, and a decline thereafter. In Singapore the incidence rate at age 65 is [illegible] cases per 100,000; in Israel the incidence rate is about 51 cases per 100,000; and in San Francisco among white females the incidence is about 156 cases per 100,000.

To elaborate on the meaning of these rates, an incidence of 23 cases per 100,000 at age 60 means that of 100,000 60-year-old women, only [illegible] develop the disease in a year's time. But it also means [illegible] that a 40-year-old woman [illegible] will have endometrial cancer [illegible]



I would like to end with some public health implications of these observations In terms of the primary prevention of endometrial cancer a program emphasizing strict weight control beginning early in life would be beneficial Obviously there are other health benefits from maintaining an ideal weight protection from endometrial cancer is just one more Second alteration of the prescribing habits for estrogens in the menopause in nonhysterectomized women is in order though undoubtedly many physicians have already done this The emphasis should be on strict indications for usage and on usage of a low dose and for a short term Theoretically estrogen progesterone combinations should be much safer and their testing should be a high priority Finally detection and correction of other hyperestrogenic states such as ovarian stromal hyperplasia would also be beneficial

In terms of the secondary prevention of endometrial cancer screening for the disease by tissue or cytologic sampling would be valuable because we know that the early cancers have an extremely good prognosis Screening is in order in certain high risk women whom we can define including the moderately and grossly obese estrogen users in the menopause those with a history of anovulation and nulliparity and those with a history of breast cancer However much remains to be done to develop a safe sensitive and specific screening test for endometrial cancer Toward this end it is hoped that new ideas and impetus will develop from this conference

Our concepts of the epidemiology of endometrial cancer have undergone some dramatic changes during the 1970s but we are entering the 1980s with a far clearer concept of its epidemiology and prevention

References

1 Armstrong B Doll R Environmental factors and cancer incidence and mortality in different countries with special reference to dietary practices Int J Cancer 15 617 1975

2 Cramer DW Knapp RC Review of epidemiologic studies of endometrial cancer and exogenous estrogen Obstet Gynecol 54 521 526 1979

3 De Vere RD Demyster KR A case of the Stein Leventhal syndrome associated with carcinoma of the endometrium J Obstet Gynaecol Br Emp 60 865-867 1953

4 Elwood JM Cole P Rothman KJ Kaplan SD Epidemiology of endometrial cancer J Natl Cancer Inst 59 1055-1060 1977

5 Jansen EI Ostergaard E Clinical studies concerning the relationship of estrogens to the development of cancer of the corpus uteri Am J Obstet Gynecol 67 1094 1102 1954

6 Jick H Watkins RN Hunter JR Dinan BJ Madsen S Rothman KJ Walker AM Replacement estrogens and endometrial cancer N Engl J Med 300 218 222 1979

7 MacMahon B Risk factors for endometrial cancer Gynecol Oncol 2 122 129 1974

8 Salerno LJ Feminizing mesenchymomas of the ovary An analysis of 28 granulosa-theca cell tumors and their relation ship to co-existent carcinoma Am J Obstet Gynecol 84 731 738 1962

9 Schoenberg BS Greenberg RA Eisenberg H Occurrence of certain multiple primary cancers in females J Natl Cancer Inst 43 15 32 1969

10 Specht H Endometrial cancer and hepatic cirrhosis Cancer 2 597-603 1949

11 Stadel BV Weiss N Characteristics of menopausal women A survey of King and Pierce Counties in Washington 1973 1974 Am J Epidemiol 102 209 216 1975

12 Walker AM Jick H Temporal and regional variation in hysterectomy rates in the United States 1970 75 Am J Epidemiol 110 41-46 1979

13 Waterhouse J Muir C Correa P Powell J (editors) Cancer Incidence in Five Continents Third volume Lyon International Agency for Research on Cancer 1976

14 Weiss NS Szekely DR Austin DF Increasing incidence of endometrial cancer in the United States N Engl J Med 294 1259 1262 1976

15 Wynder EL Escher GC Mantel N An epidemiological investigation of cancer of the endometrium Cancer 19 489 520 1966

Hormonal Characteristics of Patients with Endometrial Hyperplasia and Carcinoma (Dr Siiteri Summary Prepared by Dr Koss, with Permission)

Dr Siiteri pointed out that for many years the relationship between estrogens and endometrial carcinoma was in doubt because the disease was most frequently observed in postmenopausal women hence at a time when estrogen production was very low In younger women the endometrial disease was usually associated with abnormal patterns of ovulation such as observed in polycystic ovarian disease

In the absence of ovarian secretions of estradiol or progesterone what is the source of estrogens in such women? Current evidence points out that the adrenal glands are involved in estrogen production only indirectly They produce a precursor substance androstenedione an inactive C_{19} androgenic type compound which is converted into estrone in peripheral tissues This conversion is at a low level in normal people but may increase under certain conditions such as aging hepatic disease or obesity Similarly in polycystic ovarian disease there is an increased secretion of precursor substances that are converted into estrogens in peripheral tissues The mechanism of increased estrogen production may assume several pathways there may be an increase in the secretion of precursor substance for example from ovarian tumors or the adrenal there may be an increase in the efficiency of conversion of precursor to estrogen or there may be a modification in the mechanisms of transport of estrogens in the blood

The last factor may be related to the trace protein the sex hormone binding globulin (SHBG) SHBG has been studied previously in reference to testosterone It has been shown that the effects of testosterone are correlated not with total circulating testosterone in the blood but with the free fraction of testosterone not bound to SHBG The relationship of SHBG to estrogens was explored in studies performed in Dr Siiteri s laboratories These studies have shown that in obese women the plasma concentration of SHBG has a negative correlation with weight Particularly in postmenopausal women the body weight increment above normal has been associated with a decrease in SHBG Consequently in obese women the amount of free estrogen available for binding with target organs is increased This has been confirmed by direct measurements of free estradiol in plasma Unfortunately these differences occur in all obese women whether or not they have endometrial cancer Measurements of free estradiol in 17 women with breast cancer also disclosed the elevation of free estradiol when compared with normal well matched controls Hence the SHBG mechanism alone cannot account for endometrial carcinoma and other options need to be explored These options include other abnormal proteins other steroids and related substances that may compete with the binding of estradiol and displace estradiol to higher percentage levels These questions are being actively studied in Dr Siiteri s laboratory It is of interest from the point of view of endometrial cancer prevention that in obese women who lose weight the levels of SHBG return to normal Thus weight control may prove to be one way of reducing the risks of developing endometrial cancer

Dr Koss

The research reported by Dr Siiteri and in the next presentation would not have been possible a dozen years ago The methods of radioimmunoassay are necessary to measure and compare the very small amounts of the various hormones in the circulating blood Mount Sinai Hospital New York was in the forefront of research that has led to the development of these methods recently honored with a Nobel prize Therefore it is only fitting that the next speaker should be a member of the faculty of that institution

Sex Steroid Receptors in Endometrial Hyperplasia and Neoplasia (Dr Gurpide)

Dr Gurpide

I should start by justifying why we are talking about sex steroid receptors in a symposium on endometrial hyperplasia and cancer The justification is simple estradiol and progesterone influence the endometrium and receptors for steroid hormones are necessary for hormonal action In fact receptors are involved in all the actions of steroids There are only a few exceptions to this rule the effects of progesterone on cell membrane permeability to ions in *Xenopus* oocytes and the effects of estrogens on water imbibition in the rat uterus appear not to be mediated by receptor-hormone complexes

All the tissues that are targets for hormonal action contain receptors In fact the whole field of study of receptors began in the late 1950s when it was observed that isotopically labeled estradiol administered to rats was retained only by target tissues such as the uterus and vagina This retention was considered to be due to binding of the labeled hormone and to indicate the presence of receptors in target tissues This original postulate has been shown to be correct by accumulated evidence It was found that hormones and synthetic compounds exerting the same biologic action bind to the same receptors For instance diethylstilbestrol competes for the binding of estradiol in the uterus and is estrogenic Within any group of hormones (estrogens androgens progestins glucocorticosteroids or mineralocorticoids) the relative potency of various compounds correlates with their affinity to the receptor Furthermore there are cases of genetic deficiencies of steroid receptors that illustrate the importance of receptors in the mechanism of action of steroid hormones In the testicular feminization syndrome patients have an XY karyotype but a female phenotype in those cases the androgens produced by inguinal testes are completely inactive as a result of the absence of androgen receptors

Progesterone and estradiol are important not only in the growth and differentiation of normal endometrium but also in the development of endometrial hyperplasia and cancer Confirming results from other studies a group of investigators at King s College Hospital London recently conducted a series of experiments in which they administered estrogens to postmenopausal women in two kinds of regimens In one, they gave estrogens continuously in another they added to the estrogen regimen a progestin during one week each month With continuous estrogen treatment endometrial hyperplasia developed—in fact rather quickly The women who also received progestins showed no such effect Estrogens then induce endometrial hyperplasia which is known to be a precursor of endometrial cancer and, as Dr Cramer pointed out exogenous estrogens increase the risk for endometrial cancer Progestins can be related to this disease as both antagonists of estrogens and therapeutic agents

Since hormones require receptors to be effective it is important to look for their presence in the abnormal endometrium It is well known that the study of receptor levels in breast cancer has provided means of predicting responses of breast tumors to hormone-related treatment

In proliferative endometrium of premenopausal women there is a rather high level of estradiol receptors This level falls drastically during the luteal phase Secretory endometrium has much lower levels Progesterone may be responsible for this fall during the luteal phase since administration of Provera for two to three days to women during the follicular phase results in lower average levels of estradiol receptor than those found in the proliferative endometrium of untreated subjects This effect of progestins on estradiol receptors offers one explanation for the antiestrogenic actions of progesterone

Hyperplastic endometrium in postmenopausal women shows levels of estrogen receptors that are similar to those in proliferative endometrium These levels are higher than those of atrophic endometrium which are reported to be very low They are also higher than those of normal postmenopausal endometrium with histologic evidence of estrogenic stimulation

The average levels of estrogen receptors in well differentiated endometrial adenocarcinoma are slightly lower than those in normal proliferative endometrium Patients who have been receiving estrogens for a rather long time before diagnosis have the highest levels of endometrial estradiol receptors as if estrogens were stimulating their own receptors According to reports from research groups in Berlin and Marseilles levels of estradiol receptors may be higher in poorly differentiated than in well-differentiated adenocarcinoma On the other hand reports from Duke University and the University of Oulu in Finland lead to the opposite conclusion Although this remains a matter of dispute there is a large variance in the levels of receptors in samples of endometrial carcinoma In fact it has been reported that about 20% of the specimens of endometrial cancer do not show any detectable estradiol receptors

As I indicated progestins will lower the level of estradiol receptors in normal women The results from patients with well-differentiated endometrial cancer who received Provera for a few days also showed that in some but not all cases there is a very clear decrease in estradiol receptors Whether failure to obtain this response can be correlated with the clinical responsiveness of endometrial cancer to progestin treatment remains to be documented

The levels of progesterone receptors in normal endometrium increase during the follicular phase and peak at midcycle This pattern can be understood on the basis of two well-documented observations One is that estrogens increase the level of progesterone receptors this will account for the rise during the follicular phase The other is that progesterone itself destroys its own receptor that fact accounts for the fall in the levels during the luteal phase

In well-differentiated endometrial cancer the average level of cytoplasmic progesterone receptor is about half the average peak level seen during the normal menstrual cycle Everyone agrees that there is a fall in the levels of progesterone receptors with loss of differentiation of the tumor As observed in normal endometrium concentrations of progesterone receptors can be increased by the administration of estradiol to patients with well-differentiated endometrial cancer

Investigators at the Indiana University Medical Center are attempting to correlate the responsiveness of recurrent endometrial adenocarcinoma to progestin treatment with levels of progesterone receptors in the tissue They established a cutoff point of 50 femtomoles/mg of cytosol proteins and found that all patients with high progesterone receptor levels responded to treatment whereas only one of the ten patients with low progesterone receptor levels responded to progestin therapy These results are very interesting and the study should be extended to accumulate many more cases perhaps by the concerted efforts of several institutions

If as suggested estrogens are required to induce progesterone receptor it seems logical to attempt priming the target tissue in postmenopausal patients before looking for progesterone receptors or treating with a progestin Ethical considerations hold back studies of this type estrogen administration may be harmful to patients with endometrial cancer However a very interesting recent report from Paris indicates that tamoxifen an antiestrogen widely used in the treatment of breast cancer is a good inducer of progesterone receptors in the endometrium Therefore the administration of tamoxifen together with progestins for either diagnosis or treatment is an interesting possibility

It should be clear that the presence of receptors does not guarantee responsiveness to hormones There are many other steps involved in the mechanics of hormonal action Binding to the receptor is one activation of the receptor translocation of the receptor hormone complex to the nucleus and stimulation of transcription at the nuclear level are others Thus other tests should also be considered for the prediction of hormonal responsiveness The induction of estradiol dehydrogenase the enzyme which has been shown to be induced in the endometrium by progesterone could be a good marker for hormonal action Those tests can be done *in vivo* and perhaps *in vitro* The morphologic changes may provide the most valuable and simplest test to evaluate the responsiveness of the endometrium to hormones

In spite of the enormous amounts of beautiful work published in the last 20 years on the topic of receptors and modes of action of steroid hormones large gaps in our understanding of those mechanisms still exist particularly in relation to nuclear processes involving receptors We should then expect exciting findings from basic interdisciplinary studies on the endometrium and endometrial adenocarcinoma

Dr Koss

During the preceding part of this symposium the epidemiologic and endocrine factors of endometrial neoplasia were discussed Next we shall look at the histology and cytology of endometrial lesions The next speaker played a significant and historic role in the development of morphologic concepts of endometrial neoplasia because in 1949 he published with Dr Arthur Hertig a key paper on the lesion known today as carcinoma *in situ* of the endometrium

Histology of Endometrial Carcinoma and Its Precursors (Dr Sommers)

Dr Sommers

Endometrial adenocarcinoma is an old disease and I have seen a slide dated July 1901 from the Lenox Hill Hospital that showed a perfectly acceptable endometrial carcinoma There was a glandular component and some solid growth of epithelial cells Thus the slide showed a moderately differentiated endometrial carcinoma

I have also seen a well-differentiated or grade I endometrial adenocarcinoma from the prostate of a chromosomally normal man It was from the uterus masculinus and this is to remind you that there are at least two sites of endometrial carcinoma outside the uterine fundus the second other one is the ovary wherein an endometrioid carcinoma may occur

We recently had a case of a poorly differentiated tumor from a 21 year-old woman who never ovulated who was chromosomally abnormal with a ring marker chromosome and who had a lot of very atypical tumor giant cells This poorly differentiated or grade III adenocarcinoma was curetted on several occasions The patient could be made to cycle with estrogen and progesterone but because of gynecologic complications had a hysterectomy at age 22 She did not have a case of familial endometrial adenocarcinoma This syndrome is an important condition associated with gastrointestinal cancers in both males and females of these lineages According to Lynch s *Cancer Genetics* this group accounts for about 15% of all endometrial carcinoma cases Thus besides those mentioned by Dr Cramer women with a family history of endometrial colonic or gastric carcinoma or any combination thereof should be screened for endometrial cancer

Adenocarcinoma of the endometrium may occur in women irradiated for benign bleeding Fortunately this procedure is becoming less common Radiation is carcinogenic to the human and animal endometrium It has been said that these tumors are more often papillary but I cannot identify an endometrial carcinoma as having had a background of ionizing radiation therapy

There were 50 diabetic women who received large doses of depoestrogens to carry them through pregnancy In 1 of the 50 a hysterectomy was necessary This patient had a tremendous gross endometrial hyperplasia that on histologic examination showed a cystic glandular *proliferation and also hyperplasia* of the stroma This was an estrogen induced endometrial overgrowth Insofar as is known none of those 50 diabetic women went on to develop endometrial carcinoma Estrogen is a growth stimulant it will *prime the endometrium with hyperplasia but it takes* a mutational event a neoplastic transformation or perhaps two such events before a malignant neoplasm will occur

Dr Cramer told us about epidemiology and exposure to estrogen but this is a serious controversy and not everyone agrees with the case-control reports *Dr Alvan Feinstein an epidemiologist at Yale* pointed out in the *Journal of Clinical Diseases* the processes an individual goes through before a diagnosis of endometrial carcinoma is made He noted that there is a possibility of ascertainment bias at *each step in this series*

Controls are crucial to the validity of epidemiologic findings *The studies cited in support of the es*trogen theory of endometrial carcinoma used controls that are not regarded by Dr Feinstein and those of us who are critical of the case-control studies as making for very high grade science or general acceptability

Salmi a gynecologist in Finland writing in *Acta Obstetricia et Gynecologica Scandinavica* studied the females of the population of Turku who took estrogens She had the advantage of a registry of the entire *population of that area from which she could* pick matched controls They are the best control group that has been assembled recently The ratios of the cancer cases and the relative risk for the women developing carcinoma who took estrogens were established *The controls without cancer took more es*trogen than did the women with carcinoma of the endometrium *The conclusion of this long and splendid* review of the whole history and background of the causative factors claimed in endometrial carcinoma suggested that in Finland there is no relation between the administration of estrogen available in the same *types and doses as elsewhere* and the development of endometrial carcinoma Also in Norway Czechoslovakia and the Soviet Union three countries where estrogen is not administered endometrial carcinoma rose to the same extent during the same period as it *did in the United States*

A schema by Vellios from 1972 defined the developmental phases and morphologic changes leading to endometrial carcinoma A polyp is a focal hyperplasia that is occasionally diffuse According to Vellios the endometrium goes through sequential stages *of cystic and adenomatous hyperplasia* and dysplasia and in some cases to carcinoma *in situ* before becoming *invasive Short-circuiting is possible* and not every case goes through every step From our and others work it appears that transition from normal endometrium to carcinoma usually takes about ten years or about one-seventh of a woman s life span Cystic hyperplasia is *most common some eight to ten* years before carcinoma is found Thus it is a rather remote and unimportant precursor of endometrial carcinoma It is said that perhaps only 4% of women with cystic hyperplasia will ever develop invasive *carcinoma*

Adenomatous hyperplasia is a more closely related precursor in both time and morphology and it is most commonly observed three to five years before endometrial carcinoma However women with adenomatous hyperplasia with or without dysplasia develop carcinoma in only about 15% of cases The *other 85% never go on beyond this stage A single* curettage seems to relieve symptoms and no other therapeutic intervention is needed in approximately two thirds of the cases

What are the morphologic patterns of some of these conditions? In cystic hyperplasia the glands are spherical and lined with crowded epithelium This is important because senile atrophy has cystic glands but the lining is flat The development of cystic hyperplasia seems to require continuous proliferation *for perhaps six cycles hence approximately* four to six months The epithelium has grown equally all around the periphery of the gland the cells are lined up and palisaded and it is therefore an easily recognizable rather bland type of hyperplasia

Adenomatous hyperplasia on the other hand has outpouchings There has been irregular growth around the periphery of the endometrial glands and little buds of tissue have protruded fingerlike into the stroma This irregularity of target cell reaction is *theoretically and practically a little more sinister* However adenomatous hyperplasia does not indicate dysplasia The same kind of change occurs in polyps of the large intestine it is simply a pattern not a cytologic change In one example of adenomatous hyperplasia *showing the cloverleaf and finger*like outpouching of the glandular epithelium we saw that the cells and their nuclei and to some extent their nucleoli were enlarged the cells were irregularly piled up and all looked uniform The cells tend *to desquamate and there are cell debris in the lumens* of glands and lymphocytes or other leukocytes at tracted close to and into the glands This is helpful in distinguishing adenomatous hyperplasia from atypia or from carcinoma *in situ*

Years ago Gusberg said that *adenomatous hyperplasia* was his designation for what others called *carcinoma in situ* That was unfortunate I think highly of Dr Gusberg but he is now retired and I think maybe that concept should be retired as well

Now we come to dysplasia or atypical hyperplasia The cells are irregular in size and shape and the nuclei are irregular in size and arrangement and enlarged the nucleoli are prominent Remember that even with dysplasia and adenomatous hyperplasia only some 12% to 15% of the women will go on to develop endometrial carcinoma after a single diagnostic curettage

A historical illustration is found in *Cancer of the Uterus* by Cullen published in 1900 on cancer of the cervix and the endometrium with gross and microscopic drawings and colored illustrations In one illustration of a very hyperplastic endometrium there was a group of pale glands that differed in appearance from the hyperplastic adenomatous glands beneath Cullen said that this was the earliest stage in the evolution of cancer of the fundus and I believe he was right The lesion was rediscovered periodically for about 50 years by several other people all Hertig Bengloff and I did was give it the name *carcinoma in situ*

In one example of this lesion found incidentally in a hysterectomy specimen on the surface of the endometrium the pink glands with their pale and often slightly folded nuclei contrast considerably with the surrounding cystic hyperplastic glands The glands of carcinoma *in situ* are clean looking and the cells are alive they do not undergo pyknosis necrosis and sloughing and there is no particular attraction of lymphocytes Carcinoma *in situ* is living and growing not degenerating and sloughing

Another example of carcinoma *in situ* is characterized by acidophilic cells The nuclei are actually paler than normal This is the only carcinoma *in situ* I know of that does not show at least some nuclear hyperchromasia There is little cytologic anaplasia not much mitotic activity and by definition there can be no invasion otherwise it would just be an early endometrial adenocarcinoma The average time span between the diagnosis of carcinoma *in situ* and endometrial invasive carcinoma is about eight years Thus it is not a medical or surgical emergency In two rather young women I know of the lesion occurred in a polyp and after curettage they had successful pregnancies By itself carcinoma *in situ* is not an indication for hysterectomy

In the endometrium squamous metaplasia is not *per se* a precancerous condition Most current gynecologic pathologists agree that squamous metaplasia which may or may not be a part of chronic endometritis is not an indication of a precancerous state or of severe dysplasia

In chronic endometritis the glands are acidophilic and very irregular in both shape and cytology There are some debris in the lumens and some foci of small dark cells in the stroma When you look at these small dark cells in the stroma and hunt around you find a few plasma cells Once plasma cells are found the changes are consistent with the diagnosis of chronic endometritis and it takes most of the onus of dysplasia off the cytologic atypia The epithelial cells are atypical because of their inflamed environment and are trying to survive in a hostile situation This accounts for their ragged cell membranes for the sloughing of some of the cells and for an inability to date the endometrium as any part of a normal cycle

Hypersecretory endometrium represents a physiologic hyperplasia due to double ovulation with twice as much progesterone from two corpora lutea in the same cycle or in early as yet unrecognized pregnancy The secretory vacuoles are mostly supranuclear and we see the budding of the proliferating cells into the glands This again is not a pathologic condition

Finally the Arias Stella phenomenon is characterized by glands with peculiar large dark nuclei that stand out in a secretory or hypersecretory endometrium It indicates that somewhere in the neighborhood there is living or dying trophoblast It is not a precancerous condition but simply a marker of either intrauterine or ectopic pregnancy either current or recently aborted

Dr Koss

It should come as no surprise that the interpretation of endometrial patterns is controversial and some of Dr Sommers concepts could be the subject of a lively discussion

Cytologic Aspects of Endometrial Neoplasia (Dr Reagan)

Dr Reagan

I would like to confine my remarks to the cellular changes associated with cancer of the endometrium The cells from most endometrial cancers unlike their counterparts in the uterine cervix have a DNA content which is near normal or diploid Because of this, nuclear hyperchromasia is usually not evident Second we are dealing with very minor changes in small cells they are much more difficult to appreciate than changes in large cells Third most of the techniques accepted by the gynecologist will depend on the exfoliation of the cells and as a result we must learn to deal with very few cells If we could utilize a technique which physically removes the cells from the tumor bed we would have many more cells to work with but selling this idea to our clinical colleagues represents a major problem The features I have found to be important in the recognition of endometrial cancer are the size of the cell the size of the nucleus certain alterations in the distribution of the nuclear chromatin an increase in the size of the nucleolus and alterations in the interrelationship of the cells

We might begin by comparing four fields photographed at the same magnification and superimposed on one slide An intermediate squamous cell and its nucleus serve as a guideline for evaluating the size of the nuclei of the endometrial cells The nucleus of the intermediate squamous cell has an area of about $35 \mu m^2$ to $40 \mu m^2$ and a nucleus larger than that represents an abnormality insofar as the endometrium is concerned For comparison with the intermediate squamous cell there is a group of relatively normal endometrial cells They originated in proliferative endometrium and this in part alters the appearance of the cell The cells and nuclei are small The interrelationship of these cells is very important Normal endometrial cells are arranged like bricks in a wall they are regularly arranged in relationship to one another For comparison there is a group of cells originating in endometrial adenocarcinoma The cells are larger than their normal counterparts their nuclei are enlarged and their chromatin is aggregated Clear spaces between the chromatin aggregates may be noted The nucleoli are very slightly enlarged this is of diagnostic importance If we draw an imaginary line through the long nuclear axis and compare one cell to another there is an inconstant relationship Cells from a poorly differentiated adenocarcinoma can be recognized readily Neoplasms with this degree of differentiation are not observed very often A majority of the adenocarcinomas of the endometrium will be grade I or grade II only a minority will be poorly differentiated Your goal must be to master the detection of well-differentiated adenocarcinomas

In a well-differentiated adenocarcinoma there is often nothing in the field that enables you to gauge the size of the cell or nucleus Thus mental calibration is required It is impractical to measure each nucleus with an ocular micrometer The cells are slightly enlarged and have characteristic cytoplasmic changes their nuclei are slightly enlarged Extremely important is the change in the arrangement of nuclear chromatin The nucleoli are usually single and enlarged multiple nucleoli in these cells are not seen with any frequency The arrangement of the cells in loose clusters is typical of well-differentiated adenocarcinoma Tight aggregates of cells are not associated with this disease

In grade II adenocarcinoma the cells and their nuclei are enlarged There are prominent nucleoli and an alteration in the chromatin and one can visualize the clear interchromatinic areas In grade II adenocarcinoma one may begin to see a suggestion of a 'ball' formation because the tumor is beginning to take on a papillary growth pattern In the background of smears there is ancillary help some precipitated protein related to the fact that such patients may have a watery type of vaginal discharge

Poorly differentiated adenocarcinoma is usually identified without any problem The cells have all the features described above cell balls are now very prominent because the tumor has a dominant papillary growth pattern Poorly differentiated neoplasms are uncommon in the endometrium

The type of tumor which exists in the endometrium also has a bearing on the appearance of the cells There are basically five variants of endometrial cancer Pure glandular tumors (adenocarcinoma) make up 65% of the neoplasms Adenoacanthoma which we define as a malignant glandular tumor with a benign squamous component accounts for 19% of the neoplasms Adenosquamous cancer which we define as a neoplasm with both malignant squamous and malignant glandular components constitutes 14% of the cancers Clear cell cancer which resembles its counterpart in the cervix or vagina and secretory adenocarcinoma are unusual variants and together account for only 2% of endometrial cancers

The identification of cells from a well-differentiated adenocarcinoma may be difficult because of an excessive number of leukocytes overlying the cancer cells This is a very common problem when one deals with some forms of endometrial adenocarcinoma

In fortunate cases adenoacanthoma may be very distinctive Cells from a glandular component are accompanied by oxyphilic squamous cells they represent a specific type of metaplastic cell that is unique to the endometrium When these cells are present their origin in the endometrium is certain and there is no confusing them with their counterparts in the uterine cervix Unfortunately in many instances the metaplastic squamous cells resemble their counterparts in the cervix and it is difficult to determine the exact site of origin of such cells As a result the adenoacanthoma is often identified as an adenocarcinoma

Adenosquamous carcinoma is a tumor combining features of adenocarcinoma and squamous carcinoma I recently attended a meeting where new information was presented on this tumor It has been increasing in frequency in Japan in recent years and accounts for an increasing number of the endometrial cancers observed there A high frequency has also been reported in Australia and Hawaii In our own laboratory this tumor type accounts for more than 30% of the endometrial cancers seen in recent years The cells originating from the glandular component resemble those seen in grade II adenocarcinoma of the endometrium The cells from the squamous component resemble those originating in a large cell type of squamous cell cancer This is the most common combination observed in adenosquamous cancer of the endometrium

Clear cell carcinoma looks like its counterpart in the cervix and vagina and there is little difficulty recognizing it as adenocarcinoma The cytoplasm tends to be conspicuous or frayed and is very delicately stained perhaps this is one of the more important clues to the recognition of this tumor type

A word about the sampling methods that physically remove cells from the endometrium Many different devices are advocated for directly sampling the endometrial cavity I am concerned about this because the rules used for identifying desquamated cells do not necessarily apply to cells physically removed from their tumor bed Few laboratories in the United States have personnel competent to deal with this type of specimen I have studied this sampling technique for 25 years and while I feel that I have achieved some degree of success I have not completely mastered it

A simple way to increase the accuracy of detecting endometrial cancer is to pay attention to the presence of normal appearing endometrial cells occurring when they should not be seen This has been our experience for two consecutive five year periods In the first such period we identified 82% of the endometrial adenocarcinomas by identifying the characteristic tumor cells In that same period 12% of the patients shed endometrial cells in either the postmenopausal or perimenopausal period in the second half of the cycle On this basis these patients were investigated and one of five had a significant change in the endometrium That left us with a 6% failure to identify endometrial carcinoma Comparable results were achieved in the second five year period The accuracy achieved depends in part on the sampling method used (These values are reported in the literature) The vaginal sample alone is not the optimal one for the detection of endometrial cancer it will often be adequate however The scraping of the ectocervix is not the optimal sample either The sample obtained from the cervical canal by aspiration or swab offers a higher yield and the standard deviation is smaller indicating that the investigators have had uniformly satisfactory results With this sample adequacy is sometimes a problem meaning that a certain proportion of samples will not be suitable for examination With the Gravlee jet wash one can achieve accurate detection The problem is that some samples are limited in content collection may be painful and one cannot motivate the clinician to use this method with great frequency

In the first studies with cervical aspiration conducted in 1954 we were able to detect 90% of the lesions but about 19% of the samples were unsatisfactory for one reason or another Our current accuracy in the detection of endometrial cancer based on the use of a cervical scraping and a cervical aspiration obtained with a glass pipette is comparable for the detection of adenocarcinoma and adenoacanthoma It is higher in the detection of adenosquamous cancer The number of clear cell or secretory adenocarcinomas is too small for any meaningful conclusion Our overall accuracy is 81%

I would like to leave you with a very important point to keep in mind if you want to master endometrial cytology Remember that I have been working with this medium for 25 years and don t become discouraged if your batting average is not very good after only 2 years work

Dr Koss

Our next speaker has applied a variety of contemporary investigative techniques to the morphologic study of the endometrium

Ultrastructure and Historadioautography of Endometrial Cancer and Its Precursors (Dr Ferenczy)

Dr Ferenczy

I will present some of the results we have obtained during the past four years using various techniques including electron microscopy *in vitro* DNA histo radioautography and sex-steroid receptor measurements These techniques were performed on the same tissue specimens recovered from women with normal hyperplastic and carcinomatous endometrium

These studies were carried out in order to gain a better insight into two problems regarding endometrial hyperplasia and cancer First what is the pathogenetic relationship between hyperplasia and cancer? Second what is the relationship between their proliferation and hormonal environment? The latter consideration is important because we know that in the normal endometrium morphologic alterations are mediated by the ovarian hormones estradiol and progesterone If this is true is it possible that endometrial hyperplasia and cancer which represent abnormal endometrial proliferations are associated with an abnormal hormonal environment?

As Dr Gurpide mentioned estradiol 17β is the uterotropic hormone that produces endometrial proliferation whereas progesterone antagonizes the effect of estradiol and leads to secretory differentiation of estrogen primed endometrium DNA-historadioautography using radiothymidine during the proliferative phase of the menstrual cycle associated with high blood levels of estradiol shows massive incorporation of nucleoprotein precursor radiothymidine into the nuclei of gland cells stromal cells and vascular endothelium Since the newly formed DNA is programmed for the next phase of the cell cycle mitosis the increased uptake of radiothymidine suggests indeed that estradiol effectively promotes endometrial proliferation On the other hand the endometrium obtained during the secretory phase of the menstrual cycle (24th and 25th days) is devoid of labeled cells suggesting that no DNA synthesis takes place This is due to the fact that the endometrial glandular apparatus is not programmed for DNA synthesis or proliferation but for secretory functions presumably to receive the egg if fertilization occurs

Transmission electron microscopy of epithelial glandular cells in the proliferative phase shows a great number of organelles such as free and bound ribosomes Golgi apparatus mitochondria and membrane bound acid phosphatase rich primary lysosomes All these organelles are produced by estradiol and are programmed for glycoprotein synthesis during the postovulatory phase of the menstrual cycle At the scanning electron microscopic level the proliferative endometrium contains numerous ciliated cells alternating with nonciliated microvillous cells whereas ciliated gland cells disappear in the progestational secretory phase endometrium These observations lead to the concept that endometrial ciliogenesis as well as microvillogenesis is a reflection of the estrogenic response of the endometrium In fact they are among the most sensitive morphologic indicators of estrogenic responses as far as the endometrium is concerned

What happens in women who have only an estrogenic effect without the inhibitory action of progesterone? Of those women who have elevated estrogens of an either endogenous or exogenous source a certain proportion may respond by producing an overgrowth of the endometrium This is true for the glands the stroma and the vessels The endometrium in these conditions becomes twice or three times as thick as normal proliferative endometrium

This anovulatory persistent proliferative endometrium is essentially an exaggerated form of its normal cyclic proliferative counterpart Many investigators refer to this condition as simple hyperplasia Ultrastructurally the anovulatory endometrium contains an exaggerated number of cell organelles There is a considerable increase in the number of primary lysosomes free and bound ribosomes and Golgi apparatuses The gland cells also acquire numerous microfilaments which are oriented parallel to their long axis presumably because the cells are very tall Microfilaments at least in the endometrium are believed to serve as a supportive skeleton for the epithelial cells The nuclei have a spindle-shaped fusiform configuration and demonstrate pseudostratification

In adenomatous hyperplasia with cytologic atypia similar ultrastructural changes are noted except that the endoplasmic reticulum has a bizarre configuration and the mitochondria vary in shape and size and undergo aggregation

In carcinoma *in situ* the nuclei of gland cells demonstrate rounding of their contours and an aneuploid

DNA content The microfilaments are no longer distributed along the cytoplasmic substance but around the nuclei There is pleomorphism of the organelles especially the mitochondria Scanning electron microscopy shows a dramatic decrease in the number of ciliated cells as well as microvilli and there is an increase in cellular pleomorphism When these features are compared with those of endometrial carcinoma cells invading the myometrium the ultrastructural features are essentially the same These substructural similarities provide convincing support to light microscopy for the existence of carcinoma *in situ* of the endometrium We make the diagnosis of CIS of the endometrium when we see nuclear alterations consistent with aneuploidy and a gland in-gland or cribiform glandular pattern The general picture very closely resembles that of intraductal breast carcinoma

To further characterize hyperplastic and neoplastic endometria we have measured the duration of their DNA-synthesis (S) phase The DNA S phase lasts about seven and a half hours in normal proliferative endometrium It becomes shorter in anovulatory proliferative endometrium then increases in adenomatous hyperplasia without cytologic atypia and further increases in adenomatous hyperplasia with severe cytologic atypia There is a statistically significant increase in the duration of the DNA S phase in CIS and the CIS values are essentially the same as in invasive carcinoma This is an interesting and new observation since prolongation of the DNA S phase of any given tissue when the phase is compared with that of the adjacent hyperplastic or normal tissue is a highly specific feature of cancer in general The DNA studies therefore confirm the electron microscopic and light microscopic observations that CIS does exist Furthermore the values for adenomatous hyperplasia with severe cytologic atypia (AAH) appear to be intermediate between CIS and adenomatous hyperplasia without cytologic atypia This suggests that AAH is the precursor of CIS and that CIS in turn is the precursor of invasive cancer The ultrastructural and DNA studies obtained so far are consistent with a continuum of changes from anovulatory persistent proliferative endometrium to adenomatous hyperplasia with severe cytologic atypia and then to noninvasive and invasive carcinoma The data confirm the pathogenetic relationship between hyperplasia and cancer

The second target of our studies was the relationship between endometrial growth and hormonal environment and in particular we wished to determine the sensitivity of endometrial tissue to estradiol in various pathologic conditions Three approaches may be applied to this problem morphology measurements of DNA synthesis or of the mitotic index and measurements of receptors for estradiol and progesterone

I mentioned above that endometrial ciliogenesis and microvillogenesis were indicative of estrogenic response Anovulatory endometrium or simple hyperplasia associated with anovulation shows a dramatic increase in the number of ciliated cells and surface microvilli indicating an increased response of this type of tissue to estrogenic stimulation in the absence of detectable progesterone

In contrast adenomatous hyperplasia with severe cytologic atypia has a decreased number of ciliated cells although well-developed surface microvilli are present In CIS there is a further decrease in the number of ciliated cells and surface microvilli and in poorly differentiated adenocarcinoma ciliated cells are absent The gradual decrease in estrogen related cellular specialization is a reflection of a gradually decreasing response of these tissues to an estrogenic environment or stimulation

By using a labeling index one measures the number of cells incorporating radiothymidine The DNA synthesis values for normal proliferative endometrium are considerably decreased in atrophic endometrium In the anovulatory endometrium the values are higher than in the normal although the differences are not statistically significant There follows a dramatic decrease at the level of adenomatous hyperplasia without cytologic atypia In adenomatous hyperplasia with severe cytologic atypia in CIS and in invasive cancer however DNA synthesis is similar to that of normal proliferative endometrium

The DNA data were correlated with progesterone receptor concentrations Progesterone receptors in normal proliferative endometrium are high with concentrations of over 1 100 femtomoles/mg of protein In the atrophic endometrium receptor concentrations are very low whereas in anovulatory persistent proliferative endometrium the levels are slightly higher than in the cyclic proliferative variety Then there is a sharp but gradual decrease in adenomatous hyperplasia without and with atypia CIS and invasive carcinoma

How is all this information to be interpreted? The endometrium responds specifically to estradiol by increasing its nuclear DNA and progesterone receptors We found elevated levels of DNA synthesis in atypical hyperplastic endometrium CIS and invasive

Dr Koss

Our next speaker has applied a variety of contemporary investigative techniques to the morphologic study of the endometrium

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How is all this information to be interpreted? The endometrium responds specifically to estradiol by increasing its nuclear DNA and progesterone receptors We found elevated levels of DNA synthesis in atypical hyperplastic endometrium CIS and invasive cancer and would have expected progesterone receptors to be high also but on the contrary they are low In our opinion this dissociation between DNA synthesis (high) and progesterone receptors (low) suggests that the increased DNA synthesis in AAH CIS and invasive endometrial carcinoma is a reflection of autonomous growth and is independent of estradiol influence Moreover the logarithmic decrease in progesterone receptor concentrations from early hyperplasia to advanced hyperplasia and carcinoma reinforces the concept of an endometrial hyperplasia-carcinoma continuum

On the basis of these findings we propose that anovulatory persistent proliferative endometrium occurs following persistent anovulation It may develop in premenopausal and postmenopausal women particularly in those who are obese or receive exogenous estrogens This abnormality may persist but in our experience the anovulatory endometrium reverts to normal in 85% to 90% of cases after a thorough D & C For this reason we prefer not to use the term *hyperplasia* for this biologically labile condition Indeed in my institutions hysterectomy is the primary therapeutic approach to hyperplasia regardless of subtype Because anovulatory endometrium is easily controlled by conservative measures hysterectomy can be considered overtreatment in most of these instances

Biologically we consider anovulatory persistent proliferative endometrium as the initial, or proliferative phase of endometrial hyperplasia in which the endometrium is genuinely sensitive to estrogenic stimulation In the susceptible individual this state may progress to the second or remodeling phase of endometrial hyperplasia which is characterized by architectural and nuclear abnormalities With increasing atypical changes the endometrium becomes increasingly refractory to estradiol action Finally CIS may develop It is not known how often CIS regresses but it does represent the immediate precursor of invasive carcinoma

In summary the data available so far suggest that endometrial hyperplasia is produced at least in its early stages by an estrogenic environment in the absence of progesterone In the susceptible individual this environment may be favorable for carcinogenesis and endometrial cancer may develop Endometrial carcinogenesis *per se* is not likely to be dependent upon estrogenic stimulation

It must be recognized that histology and cytology are very subjective in the assessment of precursors and early stages of endometrial carcinoma Work such as presented by Dr Ferenczy will someday result in the objective assessment of the status of the endometrium

Detection of Endometrial Carcinoma in Asymptomatic Peri- and Postmenopausal Women (Dr Koss)

Dr Koss

I shall now summarize the present status of a project sponsored by the National Cancer Institute (Contract #NCI-CB 84233 39 from the NCI Department of Health Education and Welfare) at Montefiore Hospital and Medical Center whose purpose is the study of the detection of endometrial carcinoma in asymptomatic perimenopausal and postmenopausal women Any system of cancer detection in an asymptomatic population has several prerequisites In order to be susceptible to detection (1) a disease has to be sufficiently frequent in a population (2) it has to have identifiable precursor stages that should be amenable to treatment at low cost (3) the treatment of the precursors should lower the mortality from cancer, and (4) the available means of detection should be of high specificity sensitivity and simplicity of use

In reference to endometrial carcinoma it is important to look at the rate of lesions in a given population The rate of this disease as reported by the cancer registries of the states of Connecticut and New York increases very obviously in women age 45 and older Thus if one were to screen a population above age 45 information on the prevalence of this disease presumably could be obtained Some years ago the National Cancer Institute became interested in initiating such a detection program and we were fortunate enough to be the winning team

The purpose of what is now called the National Endometrial Program is to determine the feasibility of screening asymptomatic women age 45 and above for occult endometrial carcinoma by direct and indirect sampling techniques The prime requirement for admission to this program is a complete absence of symptoms Much of what has been published about successes in the cytologic diagnosis of endometrial cancer pertains to symptomatic women The number of endometrial cancers in asymptomatic women that have been reported in the literature to date is very small

The two commercially available endometrial sampling devices (the Mi Mark Helix Simpson/Bayse Inc Wilmington Delaware and Isaacs Endometrial Cell Sampler Kendall Corp Boston Massachusetts) that were adopted in our program by contract are assigned by computer to individual examinees In addition we also use the entire battery of the more common cytologic sampling techniques such as the vaginal pool smear and endocervical aspiration We also determine the estrogen levels in the patients by taking a scrape smear of the lateral vaginal wall From the endometrial samples we always collect miniscule fragments of tissue and embed them in paraffin as cell blocks The remaining sample is examined by cytologic techniques

I must emphasize the key roles that have been played by our project coordinators Suzanne Horan and Mary Begen R N It proved to be very important to explain the procedure to the frightened woman who comes for the examination The examinee must be reassured as to the possible complications and discomfort that may result from such a procedure and of course an informed consent form has to be signed The study incidentally is in its ninth month and the initial results presented below were tabulated a few days ago

Eight hundred ninety nine examinees were evaluated As we know the white woman is much more prone to developing endometrial carcinoma than either the black or the Hispanic woman Our population of 899 examinees was 83% white and 10% black and hence must be considered satisfactory The age distribution is equally satisfactory Seventeen percent of the women were below age 50 and 83% above

It is very important in such a study to assess whether the endometrial sampling has been successful The sampling was successful in 91% of the patients and unsuccessful in about 5% The results were uncertain in 4% Any device introduced into the endometrial cavity can cause the patient major discomfort We examined this point very carefully because it was one of the questions we attempted to answer We found that after thorough psychologic preparation of the patient it is possible to obtain an endometrial sample with relatively little discomfort in approximately 80% of patients Moderate discomfort was prevalent among these women during the procedure It is also important to ask these women 48 hours after the procedure how much pain they had and how long it lasted The majority of our patients (65%) mostly postmenopausal had no residual discomfort 27% had mild discomfort for a period of 24 hours and only very few had severe residual pain To date we have had no major clinical complications in approximately 1 000 patients

Among the first 1 000 examinees we detected 8 asymptomatic endometrial cancers all of which were proven by histology In reference to the sequence of events in the endometrium as outlined above by several speakers it is disturbing that we found very few endometrial hyperplasias in this population If one were to believe the sequence of events as described we should have found many more cases of hyperplasia than cancer It may well be that we have no talent for identifying endometrial hyperplasia in the endometrial smear or the cell blocks On rescreening this population we may find that we missed quite a few of these lesions However in the histologic material we have seen to date most endometrial carcinomas appeared as solitary lesions even though several of them were deeply invasive There was no accompanying hyperplasia in most uteri This of course casts a very interesting light on the sequence of events in endometrial carcinogenesis in the postmenopausal woman We are not trying to draw any conclusion from these observations at this time However our studies to date do not substantiate the sequence of morphologic events described previously

I would like to echo what Dr Reagan said about the interpretation of the endometrial aspiration smear Some 25 or so years ago my coworker Grace Durfee and I started looking at endometrial aspiration smears and found them very difficult to interpret The interpretation of the endometrial cytologic material in this study also proved to be extraordinarily difficult

We observed that the elderly woman often lacks medical care The first 1 000 women also harbored three low-grade precursor lesions of cervical cancer and two classical carcinomas *in situ* We identified one ovarian carcinoma in a completely asymptomatic woman and one CIS of the vulva Three carcinomas of the breast were discovered by simple palpation

It is too early to conclude whether this program is going to fulfill its promise However I am pleased that the National Cancer Institute saw fit to sponsor such a program We hope that before we embark on mass screening of our populations for endometrial cancer we will have some baseline data which are so thoroughly lacking for carcinoma of the uterine cervix

Table II *Primary Lung Cancer Cytodiagnostic Sampling Modalities with High Typing Accuracies*

Histologic diagnoses	Cytologic diagnoses	Sputum specimens	Bronchial aspirates and washings	Bronchial brushings	Fine needle aspiration biopsy smears
Epidermoid carcinoma, well differentiated keratinizing	EPIDCa	42	18	2	8
	MDCCa	5	3	1	4
	OCCa	2	—	—	—
	ADENOCa	—	1	—	—
		49	22	3	12
Epidermoid carcinoma, moderately or poorly differentiated nonkeratinizing	EPIDCa	21	6	2	2
	MDCCa	3	4	1	—
		24	10	3	0
Small cell ("oat cell") anaplastic carcinoma	OCCa	23	10	—	1
	EPIDCa PD	1	—	—	—
		24	10	—	1
Spindle-polygonal cell anaplastic carcinoma	MDCCa	4	5	—	2
	EPIDCa	—	—	—	1
	ADENOCa	2	—	—	—
		6	5	—	3
Total		103	47	6	25

EPIDCa = epidermoid carcinoma, MDCCa = medium-sized-cell (spindle-polygonal) anaplastic carcinoma, OCCa = oat cell carcinoma, LDCa = large cell anaplastic carcinoma, ADENOCa = adenocarcinoma, PD = poorly differentiated

Table III *Primary Lung Cancer Cytodiagnostic Sampling Modalities with Low Typing Accuracies*

Histologic diagnoses	Cytologic diagnoses	Sputum specimens	Bronchial aspirates and washings	Bronchial brushings	Fine needle aspiration biopsy smears
Large cell giant cell anaplastic carcinoma	MDCCa	3	2	1	5
	EPIDCa PD	4	1	—	3
	ADENOCa	—	1	—	—
	LCCa	—	—	—	2
		7	4	1	10
Bronchioloalveolar cell adenocarcinoma (3 PD + WD)	MDCCa	1	2	—	2
	ADENOCa	—	3	—	1
		1	5	—	3
Bronchogenic (acinar and papillary) adenocarcinoma (11 PD + 4 WD)	MDCCa	5	1	1	2
	ADENOCa WD	—	1	1	—
	ADENOCa PD	—	—	—	8
		5	2	2	10
Mucoepidermoid carcinoma, PD	MDCCa	—	—	—	1
Total cytologic diagnoses		13	11	3	24

LCCa = large cell anaplastic carcinoma, WD = well differentiated, other abbreviations as in Table II

carcinoma (LCCa) and medium-sized-cell anaplastic carcinoma (MDCCa) yielded a total of seven correctly typed specimens and three incorrectly typed specimens in the LCGCCa group. In the bronchioloalveolar and bronchogenic adenocarcinoma groups, typing accuracies varied from low to high, respectively.

The single case of poorly differentiated mucoepidermoid carcinoma had only one FNAB specimen revealing MDCCa cells exclusively (Table III).

When all clinical sampling methods of bronchopulmonary material were considered together with respect to the accuracy of their cytohistologic diag

Table IV Cytologic Typing Accuracy in Tumor Lung Cancer Categories Obtained When All Clinical Sampling Methods Are Combined

Histologic diagnoses	Total correct cytologic diagnoses per total specimens	Typing accuracy percentage
High typing accuracy		
Epidermoid carcinoma WD keratinizing	70/86	81
Oat cell anaplastic carcinoma	34/35	97
Spindle polygonal cell anaplastic carcinoma	11/14	79
Low typing accuracy		
Epidermoid carcinoma MD PD nonkeratinizing	31/46	66
Large cell giant cell anaplastic carcinoma	13/22	59
Bronchioloalveolar-cell carcinoma	4/9	44
Bronchogenic (acinar and papillary) adenocarcinoma	10/19	53
Total	173/23	75

WD = well differentiated MD = moderately differentiated PD = poorly differentiated

noses the results fell into two groups (Table IV)

High Typing Accuracy

In well-differentiated keratinizing epidermoid carcinoma cytologic typing accuracy reached 81% Small cell anaplastic carcinomas of the oat cell and spindle polygonal cell varieties possessed cytologic accuracies of 97% and 79% respectively

Low Typing Accuracy

In moderately differentiated nonkeratinizing epidermoid carcinoma the correct typing was noted in only 66% of the specimens In the LCGCCa group (combining the cytologic diagnoses of LCCa with the morphologically related diagnoses of MDCCa) the typing accuracy was 59% In bronchioloalveolar adenocarcinoma correct typing was noted in only 44% of the specimens whereas the bronchogenic adenocarcinoma group had a somewhat higher cytologic typing accuracy approximately 53%

Altogether 173 of 232 specimens (74.6%) were correctly cytotyped (Table IV)

Discussion

It has been stated[11,14] that about 25% of the discrepancies between the cytologic and histologic diagnoses are due mainly to the presence of poorly differentiated tumors From the data appearing in Table IV it can be noted that of a total of 232 specimens from all types of tumors 173 (74.6%) were correctly typed whereas 59 (25.4%) were incorrectly typed with respect to the histologic diagnoses

Lange and Høeg[22] reported in their study on cytologic typing of sputum and bronchoscopy material a 94% typing accuracy in well-differentiated epidermoid carcinoma 100% in adenocarcinoma 86% in small cell anaplastic carcinoma and 27% in poorly differentiated epidermoid carcinoma Of interest is their table which furnishes a comparison between previously published data by Foot[6] Sojut[2] and Hastrup[13] and those from their own study[22]

In a large series of pulmonary cancer cases detected in bronchial secretion aspirates Atay and Brandt[1] obtained cytologic typing accuracies of 92% in well differentiated epidermoid carcinoma small cell anaplastic carcinoma and well-differentiated adenocarcinoma but only 54% in large cell undifferentiated carcinoma

Associated inflammatory processes and the state of preservation of specimens may be responsible for some errors in cytologic typing of lung cancer Some authors[1,6,11,13,15,17,1,22,24] stress the following important causes of discrepancies in tumor typing of cytologic material (1) the degree and nature of the differentiation may vary significantly within the same tumor (2) necrosis which is common in the undifferentiated carcinomas (and seen quite often in sputum material) may induce tumor cells to take up nonspecific stains such as orange-G and thus appear to have undergone keratinization[17] (3) in certain poorly differentiated epidermoid carcinomas the cells may often be clustered superimposed and have nuclei of various sizes with prominent large nucleoli thus suggesting a false diagnosis of adenocarcinoma (4) the histologic as well as cytologic appearances of metastases may be different from those of the primary tumor (5) distinguishing histologically between poorly differentiated epidermoid carcinomas and some of the more anaplastic carcinomas or between the latter and poorly differentiated adenocarcinomas may often prove to be very difficult[1] and thus encountering similar difficulties in exfoliative cytology should not be surprising[1] (6) minute tissue samples of predominantly anaplastic tumors especially those obtained during fiber optic bronchoscopy render some of these tumors either undiagnosable or incorrectly diagnosable (7) some spindle polygonal cell types from small cell undifferentiated carcinomas at times may shed cells with sufficient cytoplasm to be misinterpreted as poorly differen

24 Lukeman J Reliability of cytologic diagnosis in cancer of the lung Cancer Chemother Rep 4 79-93 1973

25 ✓ Nasiell M Diagnosis of lung cancer by aspiration biopsy and a comparison between this method and exfoliative cytology Acta Cytol 11 114 119 1967

26 Reid JD Carr AH The validity and value of histological and cytological classification of lung cancer Cancer 14 673-698 1961

27 Roger V Nasiell M Linden M Enstad I Cytologic differential diagnosis of bronchiolo-alveolar carcinoma and bronchogenic adenocarcinoma Acta Cytol 20 303 307 1976

28 Smith JH Frable WJ Adenocarcinoma of the lung Cytologic correlation with histologic types Acta Cytol 18 316-320 1974

29 Spjut HJ Fier DJ Ackermann LV Exfoliative cytology and pulmonary cancer A histopathological and cytologic correlation J Thorac Surg 30 90-107 1955

30 Tsuboi E Atlas of Transbronchial Biopsy Early Diagnosis of Peripheral Pulmonary Carcinomas Stuttgart Thieme 1971 p 53

31 Wandall HH A study of neoplastic cells in sputum as a contribution to diagnosis of primary lung cancer Acta Chir Scand 91 [Suppl 93 1 143] 1944

UICC Conference on Clinical Oncology

The UICC Conference on Clinical Cytology will take place in Lausanne Switzerland October 29-31 1981 The conference is being organized by the International Union Against Cancer in conjunction with the Seventh Meeting of the European Society of Medical Oncology The conference is hosted by the Swiss Cancer League and is cosponsored by the European Organization for Research on Treatment of Cancer (EORTC) the European Association for Cancer Research and the Organization of European Cancer Institutes

This multidisciplinary conference on cancer treatment is a major innovation within the International Union Against Cancer It is open to all physicians and is designed to provide a forum for presentation of original research in all clinical aspects of multidisciplinary treatment of cancer patients Internationally renowned clinical oncologists will be invited as speakers and chairpersons Parallel sessions at which proffered papers are presented will be organized during the first part of each half day Ample time will be provided for discussions Major review lectures will be presented in plenary sessions during the second part of each half day by the chairpersons of the proffered paper sessions Poster sessions will be organized throughout the conference

Further information can be obtained from the Conference Secretariat P O Box 248 CH 1000 Lausanne 6 Switzerland

Identification of Viruses in the Urine of Renal Transplant Recipients by Cytomorphology

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This study was designed to reassess the cytomorphology of viral infections in urinary cells obtained from renal transplant patients and to examine the association if any between these cytologic changes and the transplant rejection A total of 2 354 cytologic specimens obtained from 91 renal transplant recipients was evaluated A combination of techniques including cellulosic filters immunofluorescence hemagglutination inhibition and electron microscopy was utilized Cytologic observations were correlated with the patient s clinical history Thirty eight patients revealed cytologic evidence of viral infections (herpes simplex cytomegalovirus and papovavirus) These viral infections had distinct cytomorphology Cytomegalovirus infection may manifest as intracytoplasmic orangeophilic inclusions in addition to the classical intranuclear inclusion In the majority of renal transplant patients there appeared to be no relationship between the viral infection and the renal transplant rejection episodes

Viral infections occur commonly in immunosuppressed renal transplant recipients[2,3] These infections often interfere with the clinical recognition of renal rejection episodes either by simulating the rejection itself or by masking its signs and symptoms Viruses are also suspected of initiating transplant rejection[15] Accurate and early detection of these infections in renal transplant recipients can help clinical management

Although a large number of viruses have been isolated from urine[2] the viruses most readily diagnosed by urine cytology are herpes simplex virus (HSV) cytomegalovirus (CMV) and the human papovavirus BK virus (BKV) HSV has been reported in cytologic studies of urine specimens obtained from the bladder[13,14] Its occurrence among renal transplant recipients was first documented cytologically by Bossen and Johnston[3] in 12 specimens from four patients Systemic dissemination of HSV infection with resultant death was reported by Montgomerie et al[16] CMV infection was first described cytologically in the urine of 22 renal transplant recipients studied by Bossen et al[4] The virus infected cells were characterized as having only intranuclear inclusions Their observations suggested that the cytologic technique was a more sensitive method of detecting virus in urine than was histologic examination of tissues CMV is the most frequently encountered viral infection in the urine of transplant patients[4] BKV a recently recognized human papovavirus of the SV40-polyoma subgroup[19,21] was first reported in the urine

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samples of a transplant recipient. Its cytomorphology has been described in detail by Coleman et al,[5] who have also documented its frequent reactivation in transplant patients. JC virus (JCV), another human papovavirus, has been observed recently in the urine of pregnant women.[6] JCV causes progressive multifocal leukoencephalopathy, a rare demyelinating disease of the central nervous system that occurs in immunologically compromised individuals.[21,22]

Specific identification of viruses by the classical diagnostic techniques (i.e., virus isolation, rising specific antibody titers, complement fixation test) requires considerable time and expense. Clinical cytopathology offers the possibility of a more expedient and economic method for the detection and diagnosis of these infections. The present study was undertaken to reassess the cytomorphology of HSV, CMV and papovavirus infections in urine specimens obtained from renal transplant patients and to investigate the association between the cytologic evidence of viral infection and renal transplant rejection episodes.

Materials and Methods

Patient Population

Over a seven-year period, 91 patients from the Johns Hopkins Hospital Renal Clinic were investigated clinically and cytologically. Cytologic evidence of viral infection in urine samples was recorded in 38 of these patients; they are the subject of this report. All patients were adults (18 to 60 years, mean age 40 years). The duration of the renal transplant varied from 1 day to 7.5 years (mean, 2.4 years). These 38 patients received 41 transplants, 33 from cadavers and 8 from living, related donors. A total of 2,354 posttransplant urine specimens was screened from this group.

Each patient was maintained on azathioprine (50 to 225 mg/day) and prednisone (5 to 60 mg/day). Increased dosage of prednisone and/or methylprednisolone sodium succinate, with or without extra irradiation to the graft (150 to 900 rads total dosage), was instituted during the clinical transplant rejection episodes.

Cytologic Preparation and Microscopic Evaluation

A fresh, clean-catch urine specimen was collected from each patient during the routine visit to the clinic at intervals of one to four weeks. Daily urine specimens were available for cytologic studies from inpatients. At least one cellulosic filter (Millipore Corporation, Bedford, Massachusetts, or Gelman Sciences Inc., Ann Arbor, Michigan) was prepared from 5 ml of each urine specimen. Filters were fixed in 95% ethyl alcohol and stained by a modified Papanicolaou technique.[11] Each half of the filter was screened for malignant cells and additionally evaluated for a rejection pattern[9] and for cytomorphologic evidence of the presence of virus.

Whenever cytologic viral changes were identified, a cytologic profile was determined for the specific virus-associated pattern. Twenty separate representative fields were counted (45× high dry objective) for (1) cells exhibiting viral changes, (2) tubular cells, (3) lymphocytes, (4) polymorphonuclear leukocytes and (5) various types of casts. Elements such as red blood cells, bacteria, fungi and necrosis were recorded but not quantitated.

Electron Microscopy (EM) and Immune Electron Microscopy (IEM) for Papovavirus

A total of 83 specimens was examined by electron microscopy. A 4-ml aliquot of urine was clarified by low-speed centrifugation (15 minutes, 1,000 g) and then centrifuged for one hour at 100,000 g. The well-drained pellet was resuspended and examined after negative staining for virus particles. Virions of papovavirus morphology were reacted with a BKV rabbit antiserum in an IEM test[23] and examined for antibody coating of virus particles.

Virus Isolation

An attempt was made to isolate BKV from 25 urine specimens. A 0.2-ml aliquot of undiluted urine was inoculated into each of three tubes of WI-38 cells, and the inoculated tubes were observed for viral cytopathic effect (CPE) for 30 days. Isolated strains were identified by hemagglutination inhibition (HI) tests with anti-BKV serum.[24]

Indirect Immunofluorescence on Urinary Cells for CMV

Urine samples from 18 renal transplant patients were examined. Cells from fresh, unfixed urine samples were collected by centrifugation at 6,000 rpm for five minutes. All but 2 ml of the supernatant was decanted. The cells were gently resuspended in a micropipette, placed on a cellulosic filter and fixed with 95% ethyl alcohol. Each filter was cut in half and placed cell side down on a glass slide. It was then gently but firmly blotted with coarse filter paper, using the index finger, to express moisture and to make the cells adhere to the slide The slide was immersed in cold acetone for ten minutes to dissolve the filter and then air dried The cell preparations on the slides were reacted with fluorescein isothiocyanate (FITC)-labeled specific antiserum for CMV (Microbiological Associates Bethesda Maryland) at 37 C for one hour in a moist chamber rinsed in three changes of phosphate-buffered saline solution (pH 7 2 to 7 4) for 20 minutes each and mounted in buffered glycerol Coverslips were sealed with clear fingernail polish The preparations were examined with a Leitz Orthoplan microscope equipped with Ploem illuminator KP490 BG38 exciter K530 barrier filters and HBO100 light source

Correlation of Clinical and Cytologic Observations

The patients hospital records were reviewed for dosages of azathioprine and prednisone levels of creatinine and serum urea nitrogen blood and urine cultures for virus isolations complement fixing (CF) antibody titers for HSV and CMV infection and clinical impressions about rejection episodes For each patient the clinical and cytologic data were incorporated in a histogram

Results

Of the 38 patients whose urinary cytology suggested viral infection cytomorphologic evidence of HSV infection was found in 3 (8%) CMV infection in all 38 (100%) and papovavirus infection in 24 (63%) (Table I)

HSV

A total of 157 urine samples was collected from the three patients only 8 (5%) of them showed HSV cytomorphology (Table I) Cellular changes were typical and included multinucleated syncytial formations with gelatinous ground-glass chromatinic patterns and intranuclear eosinophilic inclusions HSV infection occurred in the first 12 weeks after transplant surgery In two of the three cases there was evidence of concomitant viral infection in the lower genital tract so it is possible that the infected cells were derived from these lesions

All three patients had CF antibody titers to HSV ranging from 1 64 to 1 156 Electron microscopic examinations were performed on four of the eight cytologically positive urine specimens virus particles of herpes virus morphology were detected in two specimens one of which also contained papovavirus particles

Table I Numbers of Patients and Urine Samples Exhibiting Cytopathologic Changes of HSV CMV and BKV

Virus	Number of patients positive	Samples from positive patient	
		Total	Number positive
HSV	3 (8%)	157	8 (5%)
CMV	38 (100%)	2,334	720 (31%)
BKV	24 (63%)	1,556	199 (13%)

*Cytologic evidence of viral infection was found in a total of 38 patients

CMV

Cytomorphologic evidence of CMV infection was found at one time or another in the epithelial cells in urines of all 38 patients and in 31% of the urine samples (Table I) Cellular changes included the intracytoplasmic orangeophilic inclusions (Figures 1A and 1B) typical intranuclear inclusions (Figure 1D) or both (Figure 1C) These changes occurred most often in single cells Occasionally small tissue fragments (Figure 1D) and renal tubular casts (Figure 1E) revealed evidence of CMV infection In consecutive urine specimens from the same individual the most commonly recognizable evidence of CMV infection was the presence of intracytoplasmic inclusions These occurred as dense orangeophilic masses varying in size and shape The larger intracytoplasmic inclusions were seen in the form of bars rings or irregular cobweb shapes (Figures 1A 1B and 1C) Occasionally classical intranuclear CMV inclusions occurred in association with the intracytoplasmic orangeophilic bodies In some patients later urine sediments revealed a central well formed acidophilic or hematoxylinophilic intranuclear inclusion body surrounded by a clear halo These classical intranuclear inclusions were identified in 65 (9%) of the CMV positive specimens in urines from 22 (58%) of the 38 patients

Immunofluorescence (IF) studies were performed on cytologic preparations from 28 specimens from 18 patients They demonstrated CMV-specific antigens in the renal tubular cells and several tubular casts with cells and debris The cells were most often observed singly were somewhat enlarged and revealed granular intracytoplasmic fluorescence At times the fluorescence was more condensed and some cells contained rounded irregular clumps of intracytoplasmic immunofluorescent material (Figure 1F) corresponding to the intracytoplasmic inclusions noted on light microscopy (Figure 1G) Diffuse intracytoplasmic fluorescence was present occasionally Sometimes distinct intranuclear fluorescence was identified that corresponded to the classical intranu samples of a transplant recipient Its cytomorphology has been described in detail by Coleman et al [9] who have also documented its frequent reactivation in transplant patients JC virus (JCV) another human papovavirus has been observed recently in the urine of pregnant women [6] JCV causes progressive multifocal leukoencephalopathy a rare demyelinating disease of the central nervous system that occurs in immunologically compromised individuals [21,22]

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BKV	24 (63%)	1356	195 (13%)

*Cytomorphologic evidence of viral infection was found in a total of 38 patients

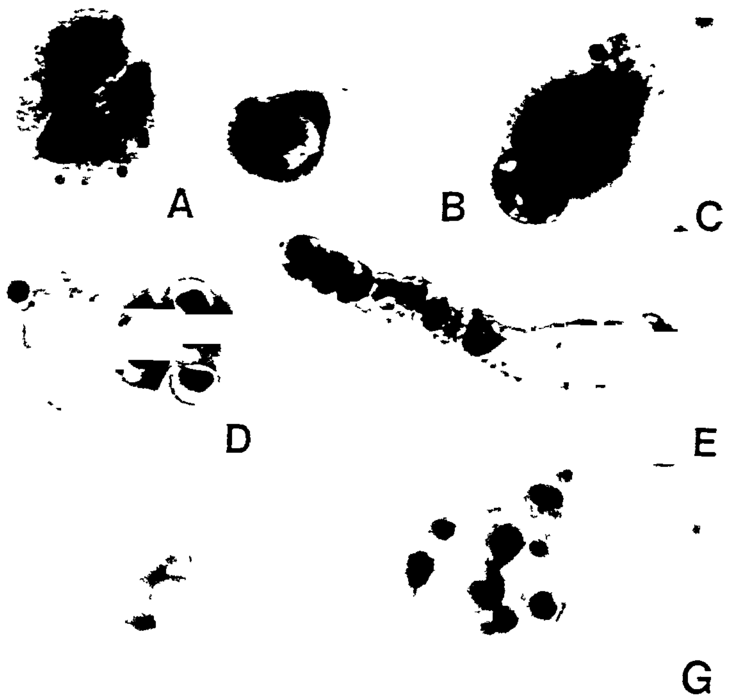

Figure 1

B. The most commonly recognized evidence of CMV in urinary epithelial cells was the presence of intracytoplasmic inclusions in the form of a [illegible] or granular shape. × 700. C. A later stage of development of CMV in urinary epithelial cells showing intracytoplasmic inclusions and a large intranuclear inclusion with associated [illegible]. × 700. D. The classical stage of development of CMV in urinary epithelial cells showing intranuclear inclusions surrounded by a clear halo. × 700. E. Occasionally [illegible] cells exhibited both intracytoplasmic and intranuclear inclusions. × 700. F. A group of urinary epithelial cells exhibiting multiple irregular clumps of intracytoplasmic [illegible] associated with CMV infection. × 700. G. The same group of cells stained with a modified Papanicolaou stain showing the corresponding intracytoplasmic inclusions associated with CMV infection. × 700.

[illegible] inclusion of CMV observed in the Papanicolaou-stained specimens. The occurrence of both intranuclear and intracytoplasmic fluorescence in the cell was rarely recognized. The diagnosis of [illegible] based on the presence of intracyto[illegible] was confirmed by IF tests 62% of the time (10 of 16) and the IF test identified CMV antigen in 16% of the cases scored as cytologically negative (2 of 12).

The time when the first urine specimen after transplantation showed cytologic evidence of CMV infection varied from 1 to 26 weeks after the transplant with a mean period of about 8 weeks The duration of shedding of infected cells was variable ranging from 1 to 41 weeks among the patients Despite the high frequency of excretion of CMV infected cells the clinical diagnosis of CMV infection was made in only one of these patients a 25 year-old male who developed prolonged fever and tenderness over the parotid area in the third week after transplantation Cytopathologic evidence of CMV infection observed mostly as irregular cytoplasmic inclusions was seen intermittently from the 6th to 32nd posttransplant week Intranuclear inclusions were seen in only one of eight urine specimens diagnosed as positive CMV was isolated from each of three specimens tested in the 7th 9th and 28th weeks The kidney removed surgically at 38 weeks contained microscopic evidence of advanced chronic rejection with superadded acute infarcts There were typical CMV inclusion bodies in the nuclei of the tubular cells (Figures 2A and 2B)

The results of other tests with respect to CMV infection were as follows CMV was isolated from blood or urine specimens in 6 of 13 patients CMV was demonstrated in histologic sections in 2 of 11 patients whose specimens were available Epithelial cells containing intranuclear inclusions characteristic of CMV were seen in the kidney of one patient and in the lungs and liver of a second patient Multiple

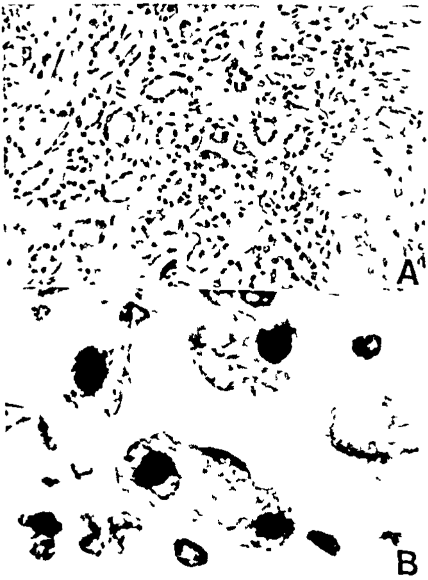

Figure 2
(A) Tissue section from a nephrectomy specimen showing evidence of CMV infection in the renal tubular epithelium and acute rejection (X 256) (B) Corresponding high-power view of renal tubular cell showing intranuclear inclusions of CMV (X 640)

serum specimens from 36 patients were tested for CF antibody titers to CMV Thirty three of the 36 patients had fourfold or greater increases in titers which were interpreted as indicating CMV infection

Papovavirus

Twenty-four patients exhibited cytomorphologic evidence of papovavirus infection observed only in single cells The most common changes were in the nucleus which occupied two thirds of the enlarged epithelial cell and whose contents became a large pale blue-green basophilic gelatinous mass (Figures 3A and 3B) Small deposits of chromatinic material were attached to the nuclear membrane and frequently the nucleus appeared to be thrusting away from the cytoplasm for a cometlike effect (*Figure 3A*) *In* some other cells generally seen in specimens obtained later during the infection the gelatinous nuclear contents became more deeply chromatophilic and appeared to shrink away from the degenerated chromatinic rim lining the nuclear membrane becoming irregularly shaped and surrounded by an indistinct halo Many times the intranuclear inclusion was attached to the nuclear membrane so that the halo was incomplete (Figures 3C and 3D) No intracytoplasmic inclusions were seen

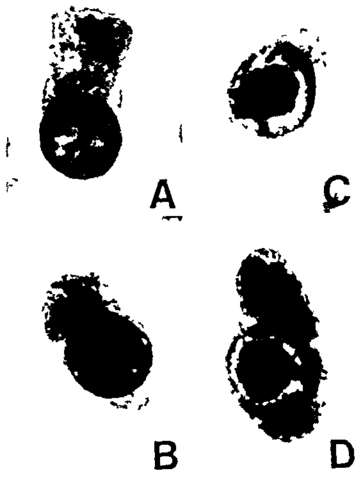

Figure 3
(A B) The most commonly recognized stage of development of BKV in urinary epithelial cells was the presence of a large nucleus occupying two thirds of the cell size and whose contents became a large gelatinous mass Note the cometlike shape of the cells (× 1 008) (C D) A later stage of development exhibited an irregular intranuclear inclusion attached to the nuclear membrane surrounded by an indistinct halo (× 1 008)

Of the 1 556 specimens from these 24 patients 198 (13%) showed cytomorphologic evidence of papovavirus (Table I) Twenty three of the patients (96%) manifested the gelatinous nuclear changes and five (21%) exhibited the nuclear changes with shrinkage into a more classical intranuclear inclusion Continuous shedding of infected cells lasted from 1 to 32 weeks with a mean of 4 8 weeks No clinical symptoms of this viral infection were evident as has also been described in earlier reports [21]

One patient with papovavirus infection developed what appeared to be a virus induced ureteral obstruction similar to that described by Coleman et al [7] The patient was a 45 year-old male who had received a cadaver kidney The first urine cytology specimens on this patient were examined in the tenth posttransplant week when nearly every cell present showed cytomorphologic evidence of either CMV or papovavirus Cells infected with CMV demonstrated a gelatinous nucleus intranuclear inclusions intracytoplasmic inclusions or both The majority of papovavirus infected cells showed intranuclear inclusions (Figures 3C and 3D) Also seen in this specimen were cells that demonstrated the cytomorphology of a gelatinous nucleus characteristic of BKV and cytoplasmic inclusions characteristic of CMV in the same cell Electron microscopy revealed a large number of papovavirus particles (Figure 4) which were identified as BKV by IEM and BKV was isolated by culture from each of two urine specimens collected over a five-day period The patient died of bilateral bacterial pneumonia and acute rejection in the 12th posttransplant week Histologic sections at autopsy revealed microscopic evidence of papovavirus infected cells in the transitional epithelium obstructing the ureter (Figures 5A and 5B) The transplanted kidney showed acute rejection In a subsequent study papovavirus antigen was identified in the urothelium of this patient by immunoperoxidase staining of a paraffin section [11]

Figure 4
Immune electron microscopic preparation showing particles identified as BKV from a patient with a virus induced ureteral obstruction (× 260 000)

Electron microscopy was performed on 83 urine samples from 21 of the 24 patients Papovavirus particles were detected in 12 samples (15%) from nine patients (43%) Viral particles in seven specimens were numerous enough for satisfactory IEM[13] in each instance the particles were identified as BKV Virus isolation was attempted from 25 urine specimens obtained from the 24 patients which included the 12 particle-positive samples BKV was isolated from each of two urine specimens examined from the patient with ureteral obstruction

Cytologic Profile

A cytologic profile was obtained on each specimen of the 38 patients in whom virus infection was suspected or diagnosed In 20 high power (40×) fields 22 patients (58%) had specimens averaging between zero and ten cells exhibiting viral changes Ten patients (26%) had 11 to 30 cells and six patients (16%) more than 30 cells exhibiting viral changes Twenty three patients (60%) shed fewer than ten renal tubular cells in 20 fields six patients (16%) were in the 11 to 30 range and nine patients (24%) had more than 30 cells The quantities and appearances of nonepithelial elements had no definite relationships to the other analyzed elements in the profile Patients who had a high count in one category did not necessarily have a high count in any of the others

Evaluation of Clinical Rejection and Presence of Virus

Cytologically viral effects could be found before during or after clinical rejection episodes Frequently patients had more than one rejection episode occurring close together The presence of virus in this instance was thought to be related to increased steroid therapy for the initial rejection Incomplete clinical data in some cases obscured the delineation of the exact beginning and end of rejection episodes No definite correlation of the cytologic presence of virus with clinical rejection was observed

Two observations however could be made First the occurrence of virus infected cells irrespective of virus type was sporadic In consecutive specimens cytopathologic viral changes were found at times in a single specimen and at other times were present in every specimen through several weeks Second during a nonrejection period serum creatinine levels had no definite association with the presence or absence of virus in exfoliated cells

Discussion

Detection and diagnosis of viral infection in renal transplant patients are important for understanding rejection mechanisms and for clinical management Urine cytology has proven to be a valuable tool in three of the common viruses affecting transplant patients HSV CMV and papovaviruses

The use of cellulosic filters and of immunofluorescence techniques aids in obtaining expedient and precise diagnoses We agree with Bossen et al[4] Johnston et al[14] and Coleman[3] that the use of cellulosic filters is the method of choice for preparation resulting in excellent cell recovery and cytologic detail for both Papanicolaou staining and immunofluorescence techniques In our experience equally good results are obtained routinely from both of the widely used cellulosic filters Millipore and Gelman

The immunofluorescence technique using cellulosic filters is particularly useful as a diagnostic tool

15 Lopez C Simmons RL Mauer SM Najarian JS Good RA Gentry S Association of renal allograft rejection with virus infections Am J Med 56 280-289 1974

16 Masukawa T Garancis JC Rytel M Mattingly RF Herpes genitalis virus isolation from human bladder urine Acta Cytol 16 416-428 1972

17 Melamed MR Wolinska WH On the significance of intracytoplasmic inclusions in the urinary sediment Am J Pathol 38 711 719 1961

18 Montgomerie JZ Becroft DMO Croxson MC Doak PB North JDK Herpes-simplex virus infection after renal transplantation Lancet 2 867-871 1969

19 Naib ZM Exfoliative Cytopathology Second edition Boston Little Brown 1976 pp 381 385

20 O'Morchoe PJ Erozan YS Cooke CR Walker WG O'Morchoe CCC Traystman MD Cowles LT Dorsch RF Frost JK Exfoliative cytology in the diagnosis of immunologic rejection in the transplanted kidney Acta Cytol 20 454-461 1976

21 Padgett BL Walker DL New human papovaviruses Prog Med Virol 22 1 35 1976

22 Padgett BL Walker DL Zukhein GM Eckroade RJ Dessel BH Cultivation of papova like virus from human brain with progressive multifocal leukoencephalopathy Lancet 1 1257 1260 1971

23 Reissig M Kelly TJ Jr Daniel RW Rangan SRS Shah KV Identification of the stumptailed virus as a new papovavirus Infect Immunol 14 225 231 1976

24 Shah KV Daniel RW Warszawski RM High prevalence of antibodies to BK virus an SV40-related papovavirus in residents of Maryland J Infect Dis 128 784 787 1973

25 Smetana H Johnston FR The origin of colloid and lipoid droplets in the epithelial cells of the renal tubules Am J Pathol 18 1029 1049 1942

26 Tweeddale DN Urinary Cytology Boston Little Brown 1977 pp 33-35

27 Utz JP Viruria in man An update Prog Med Virol 17 77 90 1974

Hogan et al (Ann Intern Med 92 373 378 1980) recently reported that JCV infections also occur frequently in renal transplant recipients

Cytopathology of Papillary Carcinoma of the Thyroid by Fine Needle Aspiration

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Joel I Hamburger M D
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[*To delineate the cytologic presentation of papillary carcinoma of the thyroid the fine needle aspiration smears were studied from 87 surgically proven cases Six diagnostically useful findings were identified papillary fronds monolayered sheets tissue fragments intranuclear cytoplasmic inclusions psammoma bodies and multinucleated giant cells*] *From 1 500 smears on cold nodules of the thyroid 6 known false negative and 4 false positive diagnoses for papillary carcinoma were made* [*The estimated accuracy of the fine needle aspirate diagnosis of papillary carcinoma is 94%*]

Recent publications in the medical literature have called attention to fine needle aspiration biopsy of the thyroid as a diagnostic adjunct to the management of patients with thyroid nodules [1-4, 11, 12] In our 26-month study of 1 500 fine needle aspirates from nonfunctioning thyroid nodules it became apparent that papillary carcinoma was the least difficult malignant thyroid disease to diagnose by this means This conclusion was based on a study of the fine needle aspirates from 87 surgically proven cases of papillary carcinoma The various cytologic changes seen in papillary carcinoma of the thyroid have been described [5-7] [This study examines the cytologic features of this cancer as to frequency diagnostic significance and differentiation from similar cytologic findings in benign disease]

Materials and Methods

[Fine needle aspiration biopsy was usually performed with a 25-gauge 1 5 inch needle and a 10-ml syringe Because papillary carcinoma is often relatively avascular a 22 20- or even 18-gauge needle was sometimes employed to obtain a satisfactory cellular aspirate The material obtained was smeared on a slide and promptly fixed with spray fixative If the initial specimen was dry a little fixative was sprayed on the subsequent slides before the material was expelled from the needle At least four and often six preparations were made from different parts of the nodule In degenerated nodules an attempt was made to obtain specimens from the subcapsular portion of the tumor The slides were stained with a modified Papanicolaou method

All the 1 500 cytologic specimens were described and then placed in one of the following categories (1) diagnostic of carcinoma (2) suspicious for carcinoma (3) indeterminate (usually a cellular follicular neoplasm) or (4) benign (nodular goiter simple adenoma and lymphocytic or granulomatous thyroiditis) An attempt was made to type the carcinomas]

All patients included in this study had either a fine needle aspiration diagnosis or a suggestion of papillary carcinoma followed by a confirmatory diagnostic lobectomy or a surgical pathologic diagnosis of papillary carcinoma preceded by fine needle aspiration diagnosis of a benign lesion

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ry tissue fragments and are pleomorphic in size and shape. Their chromatin is finely or coarsely granular. *Multiple micro- and macronucleoli are usually observed.* Intranuclear cytoplasmic inclusions are readily identified.

Psammoma Bodies

These calcific bodies have long been considered a hallmark of papillary carcinomas and are easily identified in cytologic preparations. They are formed by concentric lamellations and are found in close association with cancer cells and within tissue fragments, usually in the papillary fronds (Figure 6). Most of them are basophilic (dark blue or lavender), but some may also appear golden brown (amphophilic).

Intranuclear Cytoplasmic Inclusions

These "nuclear holes" are another characteristic feature of papillary carcinoma. They appear as sharply demarcated, unstained, round or oval areas within the nucleus. They are rimmed by condensed chromatin, and they may occupy an entire nucleus or be small and multiple (Figures 3 and 7).

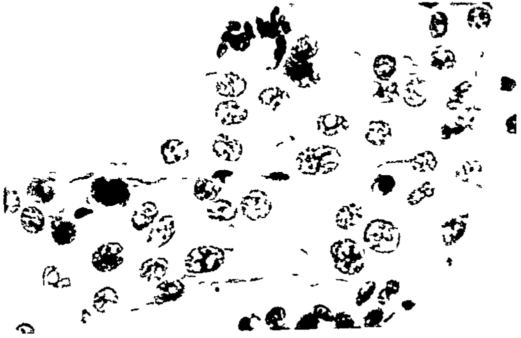

Figure 5
Papillary carcinoma. These carcinoma cells have abundant granular cytoplasm and squamoid features. Note intranuclear cytoplasmic inclusions (Papanicolaou stain, × 640).

Figure 6
Papillary carcinoma. Multiple psammoma bodies within a papillary frond (Papanicolaou stain, × 640).

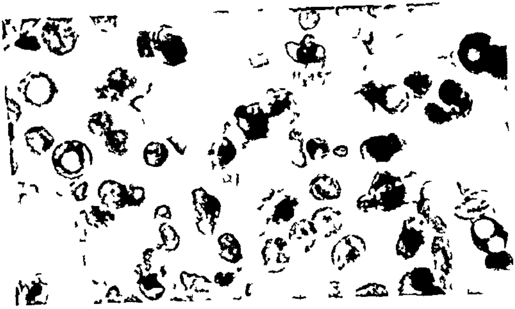

Figure 7
Papillary carcinoma Note multiple intranuclear cytoplasmic inclusions in large carcinoma cells with abundant cytoplasm (Papanicolaou stain × 640)

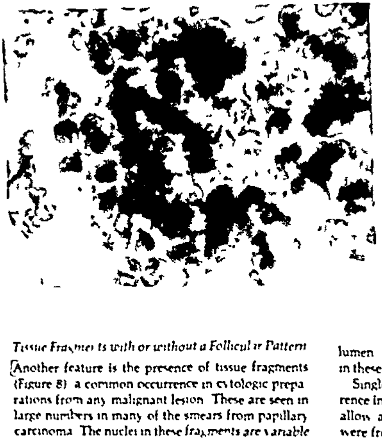

Figure 8
Papillary carcinoma A tissue fragment (Papanicolaou stain × 640)

Tissue Fragments with or without a Follicular Pattern

[Another feature is the presence of tissue fragments (Figure 8) a common occurrence in cytologic preparations from any malignant lesion These are seen in large numbers in many of the smears from papillary carcinoma The nuclei in these *fragments are variable* in size and shape depending upon the differentiation of the tumor Most papillary carcinomas have a *follicular component* (Figure 9) Small follicles with a lumen perhaps containing colloid can often be seen in these tissue fragments

Single exfoliated cells are not a common occurrence in thyroid carcinomas but when present they allow an accurate estimate of cell size]These cell were from $100\mu m^2$ to $300\mu m^2$ were variable in shape (small cuboidal columnar squamoid or polygonal and occasionally were spindle shaped (Figures 5 10 and 11)

Cytologic Diagnosis of Breast Carcinoma with Nipple Discharge

Special Significance of the Spherical Cell Cluster

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Hiroshi Yamamoto, M D
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In order to investigate the cytologic characteristics of breast carcinoma in nipple discharge 190 histologically proven cases of various breast lesions from 2 723 samples of nipple discharge were studied The general criteria of malignancy as described for other organs applied also to the breast carcinoma cells However the breast carcinoma cells were generally smaller and less pleomorphic than those arising from other organs In addition to the malignant features of individual tumor cells the presence of a spherical cell cluster with a smooth rim was also an important finding suggestive of malignancy The diagnostic rate of breast carcinoma increased significantly when the 190 cases were reexamined on the basis of the findings of this study

The incidence of breast carcinoma has been increasing annually in Japan Though a cytologic examination of nipple discharge is performed routinely spontaneous nipple discharge occurs in less than 10% of the patients with breast carcinoma Aspiration cytology of the breast has recently been put to wider use to make an early diagnosis of breast carcinoma However carcinoma cells of aspirates are somewhat different in their appearance from those of nipple discharge Therefore cytologic criteria for recognizing malignant cells in the former would be inappropriate to apply to the latter This study was attempted chiefly to further elucidate the cytologic characteristics of breast carcinoma cells in nipple discharge

Materials and Methods

From 2 723 samples of nipple discharge collected during the 12 years since the opening of the National Cancer Center Hospital 202 histologically proven cases of various breast lesions were selected for this study Of these 12 cases were excluded due to decoloration of the smears The remaining 190 cases including 62 of invasive carcinoma 18 of noninvasive carcinoma 41 of papilloma 15 of fibroadenoma 3 of cystosarcoma phyllodes 29 of mastopathy 20 of chronic mastitis and 2 of other diseases were reexamined Two slides were made routinely and stained with the Papanicolaou technique The 190 cases were divided broadly into those of carcinoma and of noncarcinoma for a careful comparative study Particularly those cells that were well preserved and adequately stained were used for a quantitative study The average frequency of cytologic examinations of nipple discharge was 2 1 times in carcinoma cases and 2 5 in noncarcinoma ones

Results

The differential cytologic findings between carcinoma and noncarcinoma cells are shown in Table I Using a micrometer the nuclear size of 2 487 carcinoma and 1 971 noncarcinoma cells was measured Among what appeared to be carcinoma cells there

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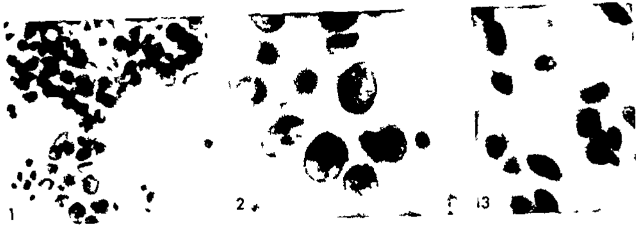

Figures 1-3
Small carcinoma cells from a case of papillary carcinoma seemingly devoid of malignant features. There were also many small pyknotic nuclei which were identified as degenerated carcinoma cells by carefully comparing discharge smears and tissue specimens as shown in Figure 4 (Figure 1 × 400, Figures 2 and 3 × 1,000). All three figures are of Papanicolaou-stained nipple discharge smears.

Table 1 *Differential Cytologic Findings between Carcinoma and Noncarcinoma in Nipple Discharge*

	Carcinoma	Noncarcinoma
Size of nucleus		
Maximum diameter		
Mean	9.4μm	7.9μm
Range	4-36μm	3-[illegible]0μm
Minimum diameter		
Mean	7.5μm	6.[illegible]μm
Range	3-27μm	2-14μm
Chromatin pattern		
Fine	20%	43%
Moderately coarse	40%	15%
Coarse	35%	35%
Other	3%	[illegible]%
Macronucleolus	[illegible]3%	<1%
Number of nucleoli		
None	45%	38%
One	44%	33%
Two	10%	[illegible]
Three or more	1.3%	1.7%
Mitosis	8.5%	[illegible]%

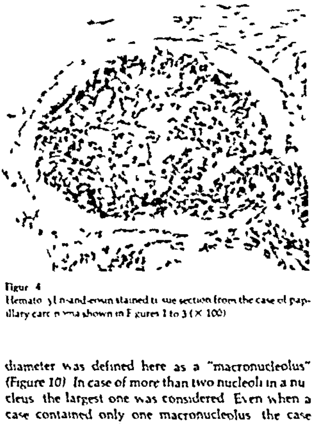

Figure 4
Hematoxylin-and-eosin stained tissue section from the case of papillary carcinoma shown in Figures 1 to 3 (× 100).

were occasional small ones not very different in size from noncarcinoma cells (Figures 1 to 4).

Chromatin was classified into four patterns: fine (Figure 5), moderately coarse (Figure 6), coarse (Figures 7 to 9) and other. The last included pyknosis, karyorrhexis and karyolysis. Moderately coarse chromatin was defined as that between fine and coarse. The two predominant chromatin patterns in each case were considered.

A nucleolus larger than one-fifth of the nuclear diameter was defined here as a "macronucleolus" (Figure 10). In case of more than two nucleoli in a nucleus, the largest one was considered. Even when a case contained only one macronucleolus, the case was counted as such. As to the number of nucleoli, the percentage of each number of nucleoli, as shown in Table 1, was based on the total numbers of carcinoma or noncarcinoma cells. There was no significant difference in the number of nucleoli between the two. A case was counted as mitotic even if only a

Acknowledgments

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References

1 Kjellgren O The cytologic diagnosis of cancer of the breast Acta Cytol 8 216-223 1964

2 Masukawa T Discovery of psammoma bodies and fungus organisms in the nipple secretion with improved breast cytology technique Acta Cytol 16 408-415 1972

3 Matsuda M Kato M Cytologic diagnosis of abnormal breast secretion J Jpn Soc Clin Cytol 8 31-44 1969 (in Japanese with English abstracts)

4 Naib ZM Exfoliative Cytopathology Second edition Boston Little Brown & Co 1976 pp 138-139

5 Papanicolaou GN Atlas of Exfoliative Cytology Cambridge Mass Harvard University Press 1963 pp 54 56

6 Papanicolaou GN Bader GM Holmquist DG Falk EA Cytologic evaluation of breast secretions *Ann NY Acad Sci* 63 1409 1421 1956

7 Papanicolaou GN Holmquist DG Bader GM Falk EA Exfoliative cytology of the human mammary gland and its value in the diagnosis of cancer and other diseases of the breast Cancer 11 377-409 1958

8 Saphir O Cytologic examination of breast secretions Am J Clin Pathol 20 1001 1010 1950

9 Vilaplana EV Jimenez Ayala M The cytologic diagnosis of breast lesions Acta Cytol 19 519 526 1975

10 Zimmerman AL King EB Barrett DL, Petrakis NL The incidence and significance of intracytoplasmic calcifications in nipple aspirate specimens Acta Cytol 21 685-692 1978

Fine Needle Aspiration Cytology of Malignant Melanoma of the Skin

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Fifty malignant melanomas of the skin were aspirated with a fine needle and the cytomorphologic features of the smears were evaluated. An attempt was made to establish the frequency of morphologic features found in melanoma cells. The following features were regarded as frequent: lack of cohesiveness between the malignant cells, binucleated, multinucleated and giant cells, and intranuclear vacuoles. The nuclei usually had a regular outline, and the nuclear chromatin was evenly distributed. The majority of the smears contained a mixture of epithelial type and spindle cells. The nuclei of the epithelial type cells were usually in an eccentric position. The morphologic features of malignant cells in smears, as described, can be helpful in making the preoperative cytologic diagnosis of nonpigmented malignant melanoma of the skin.

The clinical diagnosis of malignant melanoma of the skin is not always easy or reliable. This is due to the similarities of gross morphology of malignant melanoma and such skin lesions as teleangiectatic granuloma, basal cell carcinoma, squamous cell carcinoma, histiocytoma and sometimes even dermatofibrosarcoma or verruca seborrhoica.

There are two possible approaches to the diagnosis and treatment of malignant melanoma of the skin. One is the excision of a suspicious lesion with a narrow rim of the surrounding tissue, followed by the additional wide excision after the diagnosis of melanoma is made on frozen sections. With the other method, surgical biopsy precedes the excision of the lesion with a wide margin of surrounding tissue.

We applied fine needle aspiration biopsy as a method of preoperative diagnosis of malignant melanoma of the skin. The positive cytologic diagnosis was the basis for the excision of the lesion with a wide margin of surrounding tissue.

The cytologic diagnosis of malignant melanoma of the skin can be made from scrapings of the surface of the tumor, from smears of the ulcerated tumor or from fine needle aspirates. However, scrapings yield few cells, and they often are poorly preserved. In smears from ulcerated tumors the analysis of malignant cells is difficult because the malignant cells are often intermingled with numerous necrotic and inflammatory cells. In contrast, fine needle aspiration biopsy of skin melanoma yields a large number of well-preserved malignant cells, that makes the cytologic analysis and diagnosis easier and more reliable.

We do not believe that fine needle aspiration biopsy is more traumatic than vigorous scrapings or surgical biopsy. The old notion that diagnostic surgical biopsy can cause the spread of the malignant melanoma cells is not supported by recent opinions.[4-6,8,11-16]

In this report we analyze the morphologic features of malignant cells that can be useful in the cytodiagnosis of malignant melanomas of the skin. To the best of our knowledge, this is the first published report on the application of fine needle aspiration biopsy to the diagnosis of these tumors.

Materials and Methods

Fifty patients with malignant melanoma of the skin were studied. There were 47 primary tumors and 3 recurrent nodules in the vicinity of previously removed primary skin lesions. Clinically these were

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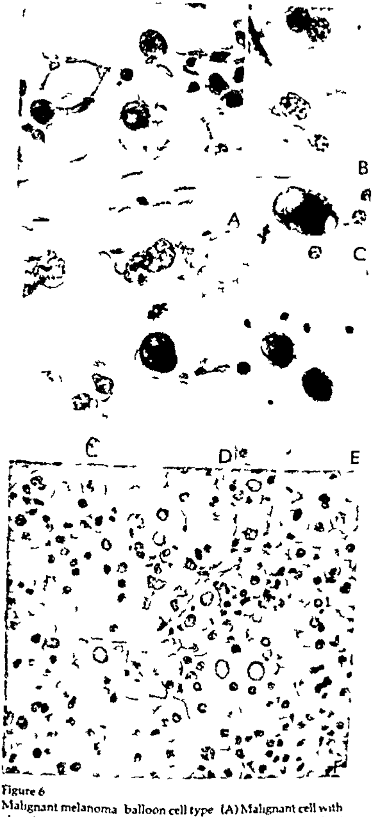


Figure 6
Malignant melanoma balloon cell type (A) Malignant cell with abundant vacuolated cytoplasm and an intranuclear "vacuole" (× 500) (B) Binucleated cell with abundant vacuolated cytoplasm and one spindle-shaped cell (× 500) (C) Several intranuclear "vacuoles" (× 500) (D) Two small groups of cells with vacuolated cytoplasm Note the multinucleated cell and mitotic figure (× 500) (E) Binucleated cell with elongated eosinophilic cytoplasm and the intranuclear "vacuole" (× 200) (F) Histology of the same tumor (× 200)

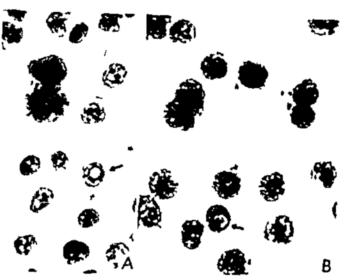


Figure 7
Malignant melanoma epithelial cell type (medium-sized cells) (A) Note the irregular nuclear contour Nuclear vacuoles (arrows) One large binucleated cell (× 500) (B) MGG-stained smear from the same tumor as that in Figure 9A *Note the intranuclear "vacuole" (arrow)* (× 500)

ular and evenly distributed throughout the nucleus in both the epithelial type and spindle cells

The majority of cells in all smears had one two or three nucleoli They were small or medium sized In 6 of 50 cases numerous cells had nucleoli larger than one-quarter of the diameter of the nucleus In four of them the large nucleoli were round and regular in outline (Figures 2A 2C and 2D) and in the remaining two the large nucleoli were irregular

Intranuclear vacuoles were usually sharply defined regular in outline round or ovoid and faintly pink stained (Figures 1A 1B 2D to 2F 5B 6C 6E and 8B) In some cells they were very large and surrounded by a narrow rim of nuclear chromatin only (Figure 2E) Sometimes they were so tiny that they could be seen only under high magnification In some cells there were several vacuoles in one nucleus (Figure 6C) Intranuclear vacuoles were present in 42 of 50 smears They were numerous in 12 cases rare in 24 and infrequent in 6 They were more frequent in the epithelial type cells than in the spindle cells Four of the eight cases without intranuclear vacuoles were of the spindle cell type In the malignant cells stained with MGG the intranuclear vacuoles could also be identified (Figure 7)

Under the electron microscope the vacuoles constituted regular oval cytoplasmic inclusions inside the nucleus They were separated from the chromatin by a continuous nuclear membrane They contained cytoplasmic organelles i e mitochondria mela

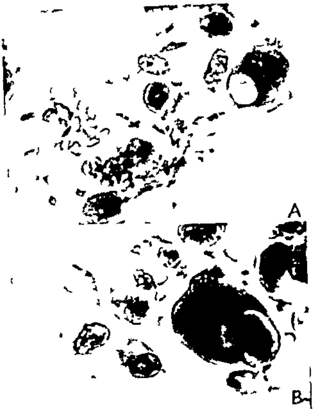


Figure 8
Melanoma malignum, mixed epithelial and spindle cell type. (A) Two multinucleated cells, one with large intracytoplasmic vacuoles (× 500). (B) Large multinucleated cell with intranuclear vacuoles and with phagocytic cell image (cell-in-cell) (× 500).

nomes and ergastroplasm and sometimes myelin figures (Figure 9A). In some nuclei several small cytoplasmic inclusions were present (Figure 9B). However, *in a few nuclei small areas of low electron density* were present without any demarcation from the surrounding chromatin (Figure 9C). We believe that these small areas are different from the sharply outlined vacuoles observed in cytologic smears.

Binucleated malignant cells were present in all smears. They were numerous in 31 cases, rare in 16 and occasional in 3 (Figures 2A, 2B, 3C, 4A, 4B, 5A to 5C, 7A, 7B, 8A and 10A). The nuclei of the binucleated cells of the epithelial type and small cell type were located at the periphery of the cell (Figures 2A, 2B, 5A and 5B). Usually the nuclei were in contact with each other; sometimes they partially overlapped, but without molding. The nuclei in the spindle cells were usually placed in a row along the long axis *of the cell (Figure 3C).* Rarely, they were found side by side (Figure 10A).

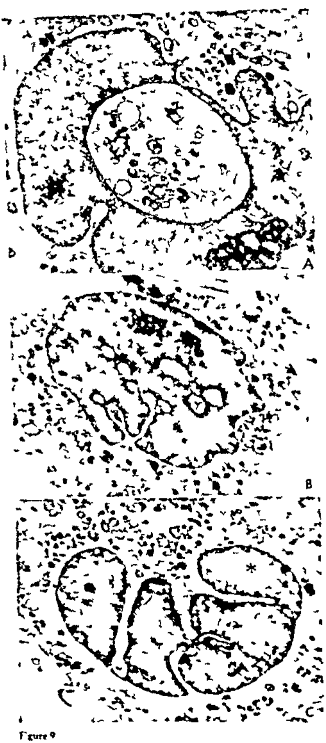
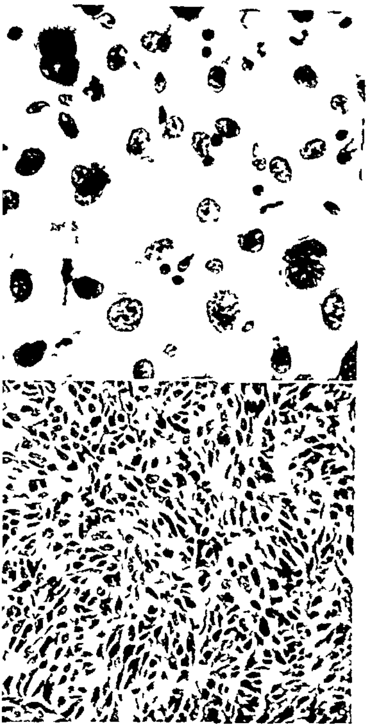


Figure 9
Electron micrographs of malignant melanoma cells. (A) Large intranuclear cytoplasmic inclusion separated from the chromatin by the continuous double nuclear membrane (× 16 000). (B) Several small intranuclear inclusions, some of them having a form of deep cytoplasmic invaginations (× 6 000). (C) Intranuclear area of low electron density with few dispersed granules of chromatin (*). This area is not surrounded by any membrane. Several intranuclear cytoplasmic invaginations are also present (× 10 000).



gure 10
lalignant melanoma mixed epithelial and spindle cell type (A)
olated cells with oval polygonal or spindle-shaped cytoplasm
uclei with finely or coarsely granular chromatin Many large
lls some of them binucleated (× 500) (B) Histology of the same
imor (× 200)

Multinucleated cells were found in 32 of 50 cases
hey were rare in 23 and occasional in 7 In two cases
f giant cell melanoma the multinucleated cells were
ı the majority The multinucleated cells were usual
y larger than mononuclear melanoma cells They
ad three to ten nuclei of various sizes and chromatin structures (Figures 4A 4B 6C and 8A) There was ı
nuclear molding Sometimes in the multinucleat
cells intranuclear vacuoles were found in only one
the nuclei (Figure 8B)

Cytoplasmic Vacuoles Intracytoplasmic vacuol
were seen in only 2 of 50 cases (Figure 8A)

Large Cells Cells four or more times larger than th
average cell in the smear were called 'large cells
They had one or multiple nuclei *Smears of 45 of 5*
of our cases contained large cells They were fre
quent in 9 cases rare in 23 and occasional in 13 (Fig
ures *1B* inset *5B, 5C 8A 8B* and 10A)

Mitotic Figures Mitotic figures were found in 31 o
50 smears They were frequent in 9 rare in 18 an
occasional in 4 Atypical mitotic figures were occa
sionally found in *27* of 50 cases (Figure *1B)*

Phagocytic Image of Cells Malignant cells with a
phagocytic image (cell in cell) were occasionally ob
served in 3 of the 50 cases (Figures 2C and 8B)

Necrotic Cells Cell necrosis and pyknosis were not observed

Inflammatory Cells and Macrophages We did not find *a significant number of lymphocytes or plasma* cells in our material A small percentage of lymphocytes and neutrophils in smears was considered to be part of the aspirated blood Rarely there were macrophages containing melanin granules or fragments *of cells in their cytoplasm*

In many cases the careful analysis of cytologic features allowed us to establish the diagnosis of malignant melanoma of the skin in the absence of melanin pigment Comparison of cytology and histology showed that size and shape of cells in smears and corresponding histologic sections of the same tumor were almost identical (Figures 2 to 6 10) However the melanin pigment *was found more often and easily* in histologic sections

Discussion

To a certain degree the morphology of melanoma cells depends on the fixation and staining of smears For instance the intranuclear vacuoles have not been mentioned by authors using MGG staining [12,17,21] However it is not unlikely that some of the intranuclear vacuoles in MGG-stained smears were falsely considered huge nucleoli [3]

The intranuclear vacuoles in the malignant melanoma cells were described for the first time by Apitz [1] who thought they were involved in melanogenesis Our electron microscopic study confirmed the previous ultrastructural observations[20,21] that these vacuoles are intranuclear cytoplasmic invaginations

Yamada et al[33] pointed out that the intranuclear vacuoles are quite valuable in the identification of melanoma cells in smears We found intranuclear vacuoles in 42 of 50 cases They are an important but not crucial diagnostic feature of malignant melanoma cells They are sometimes hardly visible in smears composed mainly of spindle cells On the other hand similar vacuoles can be found in cells of papillary thyroid carcinoma [15,22] meningioma [31] bronchogenic adenocarcinoma[10] and neurofibroma[14] as well as in some benign cells such as liver cells[21] and renal tubular epithelial cells [21]

The cytology of melanoma depends also partially on the site of the tumor and how the cells were obtained There have been several reports on the cytologic features of malignant melanomas in effusions [7,10,23] cerebrospinal fluid [10,23] urinary sediment [10,18] sputum [7,10] smears from the surface of tumors of the skin[9,24,25,27] and mucous membranes of the mouth [32] stomach [16] esophagus[2] and cervix[11,2] as well as dab smears from the cut surface of the surgically removed tumors [3,32] The cytologic features of malignant melanoma metastatic to the lymph nodes have also been described [12,16,33] and thin needle aspiration biopsy as a method of preoperative cytodiagnosis of some malignant melanomas of the skin without ulceration is mentioned in two textbooks [17,28]

The smears from the surface of the ulcerated tumors often contained large clumps of melanoma cells contrary to the dissociated cells in thin needle aspirations The spindle cells were not observed in effusions [10,23] Increases in the nuclear-cytoplasmic ratio [4] nuclear hyperchromatism [27] giant nucleoli [29] irregular nuclear outlines and phagocytic cell images [1] which have been described by some authors as frequent or characteristic features of malignant melanoma cells were not confirmed in our material

Cytologic features of melanoma cells frequently seen in our material and that also have been described as such by others are eccentrically located nuclei [13] bi and multinucleated cells [7,13] large cells regular nuclear outlines [31] evenly distributed chromatin [2] lack of cellular cohesiveness [31] epithelial-type cells mixed with spindle cells[17,24,25] and intranuclear vacuoles [33] These features greatly facilitate the cytodiagnosis of malignant melanoma in smears without melanin pigment in the cytoplasm of malignant cells In the majority of our cases these features made it possible to differentiate malignant melanoma of the skin from the other most commonly encountered skin tumors However except for melanin pigment none of the above listed cytologic features is specific for malignant melanoma of the skin and none except binucleated cells was present in the smears from all our cases

References

1 Apitz K Über die Pigmentbildung in dem Zellkernen melanotischen Geschwulste I Beitrag zur Pathologie des Zell Kerns Virchows Archiv 300 89 112 1937

2 Broderick PA Allegra SR Corvese N Primary malignant melanoma of the oesophagus A case report Acta Cytol 16 159 164 1972

3 Castelain G Castelain C Auperin R Cytodiagnostic extemporane et cytopronostic des tumores pigmentees de la peau Presse Med 68 1210-1212 1960

4 Cranmer LG Biopsy of malignant melanoma JAMA 239 9 8 1978

5 Ehrmann RL Younger PA Lerch VL The exfoliative cytology and histogenesis of an early primary malignant melanoma of the vagina Acta Cytol 6 245 254 1962

6 Epstein E Bragg K Linden G Biopsy and prognosis of malignant melanoma JAMA 208 1369 1371 1969

7 Hajdu SI Savino A Cytologic diagnosis of malignant melanoma Acta Cytol 17 3 0-3 7 1973

8 Hering H Wert der Zelldiagnose beim malignen Melanom Dermatol Monatsschr 156 385 388 1970

9 Kopf AW Mintzis M Bort RS Diagnostic accuracy in malignant melanoma Arch Dermatol 111 1291 1975

10 Koss LG Diagnostic Cytology and Its Histopathologic Bases Third edition Philadelphia JP Lippincott 1979 pp 671 955 956 978

11 Linthicum CM Primary malignant melanoma of the vagina A case report Acta Cytol 15 179 181 1971

12 Lopes Cardozo P Atlas of Clinical Cytology Hertogenbosch Targa b v 1976 pp 5 8-613

13 Meyerson LB Biopsy of malignant melanoma JAMA 239 9 8 9 9 1978

14 Müller W Dahmen HG A combined neurofibroma-granular cell tumor of middle cranial fossa Pathol Res Pract 163 37 386 1978

15 Olszewski W Woyke S Aspiration cytodiagnosis of thyroid tumors Pathol Pol 9 467-4 4 1978 (in Polish summary in English)

16 Pereira FA Biopsy of melanoma JAMA 4 434 1976

17 Pietrowa AS Prochowa MP Rukowodstwo po cytologiczeskoj diagnostike opucholej czelowieka Moscow Medicina 1976 pp 2 6-2 8

18 F a A Koss LG Cytologic diagnosis of metastatic malignant melanoma in urinary sediment Acta Cytol 8 3 4 7 1964

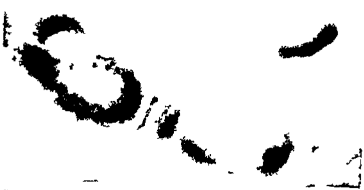


Figure 1
Ova of S stercoralis in Papanicolaou smear of duodenal aspirate (original magnification × 600)

Figure 2
Larva of *S stercoralis* in Papanicolaou smear of duodenal aspirate (original magnification × 600)

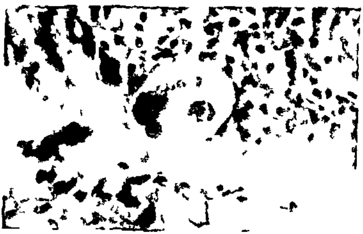


Figure 3
Ovum of *S stercoralis* in duodenal biopsy (hematoxylin and eosin original magnification × 600)

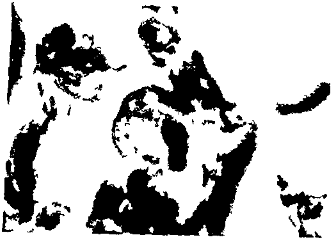


Figure 4
Larvae of *S stercoralis* in duodenal biopsy (hematoxylin and eosin original magnification × 600)

Follow up

The patient continued to have vomiting and diarrhea throughout the hospital course He died on the third day after starting thiabendazole therapy Autopsy *was not permitted*

Discussion

Ova of *Strongyloides stercoralis* are so well developed when first discharged by the adult female in the submucosa of the gut that by the time they reach the lumen they contain fully developed larvae or have already hatched The ova and *larvae* therefore may be obtained in a gastroduodenal aspirate but usually *only larvae are encountered in the feces*

Ova of *Ancylostoma duodenale* may be associated with ova and larvae of *Strongyloides* in a gastric or duodenal aspirate They have a similar hyaline transparent egg shell which is only slightly larger (60μm × 40μm) than that of *Strongyloides* They are easily differentiated from those of *Strongyloides* since they *are much less developed containing only few cells or* maximally a morula stage In the rare instance of severe constipation they may develop a larva but this would happen in the lower intestine and the possibility of obtaining them in a gastroduodenal aspirate is very far fetched Eggs of *Ancylostoma* do not hatch in the human intestine

In cultures of diagnostic material from a patient with ova of both parasites rhabditiform and filariform larvae of both nematodes and free living adults of *Strongyloides* may be encountered

A rhabditiform larva is so named because of the characteristic shape of its esophagus i e a club shaped anterior portion postmedian constriction and posterior bulbus. This esophagus occupies the anterior third of the digestive tract and is followed by a midgut and a short rectum. Rhabditiform larvae of *Strongyloides* have a short, narrow buccal channel (occupying the interval between the anterior end of the larva and the anterior end of the esophagus), a blunt tail tip (the part of the larva behind the anus) and a large genital primordium (midway the length of the midgut), in contrast to those of *Ancylostoma* larvae, which have a long, narrow buccal channel, a sharp tail tip and a small genital primordium (Table I).

A filariform larva is so named because of a characteristic club-shaped esophagus. This is followed by a midgut and rectum. Filariform larvae of *Strongyloides* have a characteristically long esophagus, constituting one half the length of the gut, and a notched tail tip, in contrast to those of *Ancylostoma* larvae, which have a remnant of esophagus about one fourth the length of the gut and a pointed tail (Table II).

Infection of this patient with *S. stercoralis* most probably took place in Jamaica and was perpetuated by autoinfection for over 50 years despite the lack of reexposure. Infection with *S. stercoralis* takes place by the filariform larvae penetrating the skin, rarely the mucous membranes of the mouth, and gaining access to the venules. Traversing the venous circulation to the right side of the heart, the parasites are carried to the pulmonary circulation, where they break through the pulmonary capillaries into the alveoli.

While in the lung, they molt. If the reaction of the host is so intense as to delay the passage of the larvae, they become adults, and the fertilized females colonize the bronchial tree and produce ova and larvae, which may be detected in the sputum.[19,19,32] Wandering larvae may reach the pleural or pericardial cavities, where they may be detected in effusions. Some larvae pass through the pulmonary capillaries to the left side of the heart, to be distributed to all body tissues. Remarkable among these is genitourinary strongyloidiasis,[10] where larvae may be detected in the urine.[10] Strongyloidiasis of the nervous system is well known.[30] Reports of *Strongyloides* larvae in Papanicolaou smears of pericardial and pleural effusions, urine and cerebrospinal fluid of infected patients have yet to be published.

The majority of larvae migrating through the lung pass up the bronchial tree to the larynx, where they are swallowed down to the small bowel. Females are fertilized and penetrate the epithelium to the mucosa, where they lay their ova. Rhabditiform larvae hatch out and pass through the mucosa into the intestinal lumen, where they pass down the stream to the soil.

Internal autoinfection occurs when the bowel stream is delayed, allowing rhabditiform larvae, while still in the intestinal lumen, to change to filariform larvae, which penetrate the intestinal wall to the capillaries and start circulating in the body.[10] In patients with very low resistance, rhabditiform larvae may induce such infection.[10] Filariform larvae on the perineal skin may induce an external autoinfection.[10]

In the soil, rhabditiform larvae molt and change to adults. Ova and new rhabditiform larvae are produced as long as environmental conditions are favorable. Otherwise rhabditiform larvae change to filariform larvae, which penetrate the skin of a new host.

Eosinophilia is usual in strongyloidiasis, and its absence in this patient indicated a poor prognosis. The fatal issue of this case may be attributed to old age, recent surgery,[31] anemia and inanition, factors that lower the resistance and increase susceptibility to hyperinfection and disseminated strongyloidiasis.[30] However, this last condition could not be substantiated since an autopsy was not done.

The laboratory diagnosis of *S. stercoralis* is usually done by detection of rhabditiform larvae in fresh fecal smear or after concentration. Rhabditiform and filariform larvae and adults could be detected after culture of feces.[4] Rhabditiform larvae could be

Table I Differential Features of Rhabditiform Larvae of *Strongyloides* and *Ancylostoma*

	Strongyloides	*Ancylostoma*
Size average	5μm × 16μm	5μm × 1[illegible] m
Anterior end	Short narrow buccal cavity	Long narrow buccal cavity
Tail tip	Blunt	Sharp
Genital primordium	Large, on ventral side halfway down midgut	Small

Table II Differential Features of Filariform Larvae of *Strongyloides* and *Ancylostoma*

	Strongyloides	*Ancylostoma*
Tail tip	Notched tail	Pointed
Esophagus length	One half esophagus length of gut	Remnant of esophagus one fourth gut

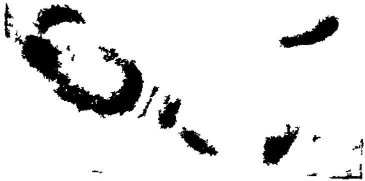
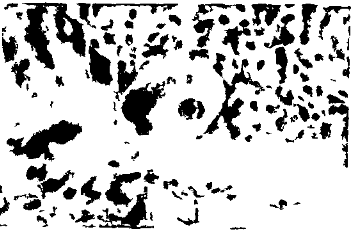


Figure 1
Ova of *S. stercoralis* in Papanicolaou smear of duodenal aspirate (original magnification × 600)

igure 2
arva of *S. stercoralis* in Papanicolaou smear of duodenal aspirate riginal magnification × 600)



gure 3
um of *S. stercoralis* in duodenal biopsy (hematoxylin and sin, original magnification × 600)

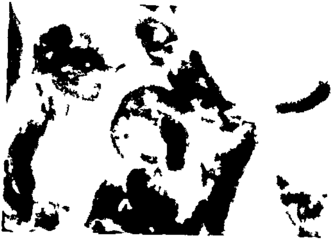


Figure 4
Larvae of *S. stercoralis* in duodenal biopsy (hematoxylin and eosin, original magnification × 600)

Follow up

The patient continued to have vomiting and diarrhea throughout the hospital course. He died on the third day after starting thiabendazole therapy. Autopsy was not permitted.

Discussion

Ova of *Strongyloides stercoralis* are so well developed when first discharged by the adult female in the submucosa of the gut that by the time they reach the lumen they contain fully developed larvae or have already hatched. The ova and larvae, therefore, may be obtained in a gastroduodenal aspirate, but usually only larvae are encountered in the feces.

Ova of *Ancylostoma duodenale* may be associated with ova and larvae of *Strongyloides* in a gastric or duodenal aspirate. They have a similar hyaline transparent egg shell, which is only slightly larger (60µm × 40µm) than that of *Strongyloides*. They are easily differentiated from those of *Strongyloides* since they are much less developed, containing only few cells or maximally a morula stage. In the rare instance of severe constipation, they may develop a larva, but this would happen in the lower intestine and the possibility of obtaining them in a gastroduodenal aspirate is very far fetched. Eggs of *Ancylostoma* do not hatch in the human intestine.

In cultures of diagnostic material from a patient with ova of both parasites, rhabditiform and filariform larvae of both nematodes and free-living adults of *Strongyloides* may be encountered.

A rhabditiform larva is so named because of the characteristic shape of its esophagus, i.e., a club-shaped anterior portion, postmedian constriction and posterior bulbus. This esophagus occupies the anterior third of the digestive tract and is followed by a midgut and a short rectum. Rhabditiform larvae of *Strongyloides* have a short, narrow buccal channel (occupying the interval between the anterior end of the larva and the anterior end of the esophagus), a blunt tail tip (the part of the larva behind the anus) and a large genital primordium (midway the length of the midgut), in contrast to those of *Ancylostoma* larvae which have a long, narrow buccal channel, a sharp tail tip and a small genital primordium (Table I).

A filariform larva is so named because of a characteristic club-shaped esophagus. This is followed by a midgut and rectum. Filariform larvae of *Strongyloides* have a characteristically long esophagus constituting one half the length of the gut and a notched tail tip, in contrast to those of *Ancylostoma* larvae which have a remnant of esophagus about one fourth the length of the gut and a pointed tail (Table II).

Infection of this patient with *S. stercoralis* most probably took place in Jamaica and was perpetuated by autoinfection for over 50 years despite the lack of reexposure. Infection with *S. stercoralis* takes place by the filariform larvae penetrating the skin, rarely the mucous membranes of the mouth, and gaining access to the venules. Traversing the venous circulation to the right side of the heart, the parasites are carried to the pulmonary circulation, where they break through the pulmonary capillaries into the alveoli.

While in the lung they molt. If the reaction of the host is so intense as to delay the passage of the larvae, they become adults and the fertilized females colonize the bronchial tree and produce ova and larvae, which may be detected in the sputum.[illegible] Wandering larvae may reach the pleural or pericardial cavities, where they may be detected in effusions. Some larvae pass through the pulmonary capillaries to the left side of the heart, to be distributed to all body tissues. Remarkable among these is genitourinary strongyloidiasis,[19] where larvae may be detected in the urine.[19] Strongyloidiasis of the nervous system is well known.[30] Reports of *Strongyloides* larvae in Papanicolaou smears of pericardial and pleural effusions, urine and cerebrospinal fluid of infected patients have yet to be published.

The majority of larvae migrating through the lungs pass up the bronchial tree to the larynx, where they are swallowed down to the small bowel. Females are fertilized and penetrate the epithel[illegible] where they lay their ova. Rhabd[illegible] out and pass through the mucosa [illegible] lumen, where they pass down the [illegible]

Internal autoinfection [illegible] stream is delayed, al[illegible] while still in the [illegible] form larvae, which [illegible] the capillaries and [illegible] patients with [illegible] vae may [illegible] the [illegible] tion.[illegible]

In [illegible]

[illegible]

Table I. D[illegible] of Rhabditiform Larvae of Strongyloides [illegible] Ancylostoma

	S[illegible]	[illegible]
S[illegible]	[illegible]5μm × 16μm	[illegible]5μm × [illegible]μm
Anterior end	Short narrow buccal cavity	Long narrow buccal cavity
Tail tip	Blunt	Sharp
Genital primordium	Large [illegible] midway down midgut	Small

Table II. D[illegible] of F[illegible] Larvae [illegible] [illegible] Ancylostoma

	S[illegible]	[illegible]
Tail [illegible]	[illegible]	[illegible]
Esophagus [illegible]	One half [illegible]	[illegible]

found in duodenal aspirate even when stools are negative[10] This is the first report in which ova of *S stercoralis* were detected in a Papanicolaou smear of gastric aspirate There is one report of the larvae of this parasite in gastric and duodenal aspirates[33] and three reports of them in the sputum[18,19,32] The gastric *aspirate of this patient was sent for cytology to con*firm or exclude malignancy of the GI tract which was the patient s admission diagnosis Although this was the primary goal of cytology it is clear that parasitic infections imposed themselves on the cytodiagnosis

The cytopathologist should become thoroughly acquainted with the life cycle of parasites inside the human body to predict their existence in the various tissues secretions and exudates This laboratory is not unfamiliar with parasites in Papanicolaou smears a case of hydatid sand was reported from an aspirate of a liver hydatid cyst[13] There is already a list of protozoa[8,14,15,23,28,29,35,37,42] trematodes[2,4,6,27,39,41,44] hydatid sand[1,9,13,16,24,38] and nematodes[5,7,12,18,22,25,32,35,40,42,43,45] reported in Papanicolaou smears from various sources in the body (Tables III to VI) *Demodex folliculorum* and *Acarina* were also reported[11,26] References to *T vaginalis* ova of *Schistosoma* and *Enterobius* and *G lamblia* could be found in a standard book of cytologic diagnosis[20] The list of parasites found in Papanicolaou smears will certainly grow

Table III *Protozoa Other Than* T vaginalis *Reported in Papanicolaou Smears*

Organism	Source	Authors	Year
E histolytica	Vagina & cervix	Munguia et al[3]	1966
	Colonic washings	Clark et al	1972
	Vagina	Torres & Benitez Bribiesca[1]	1973
Entamoeba species undecided	Vagina & cervix	McNeil & de Moraes Ruehsen	1978
E gingivalis	Sputum	Rosenberg & Rachman	1970
Acanthamoeba	GI washings	Hoffler & Rubell	1974
G lamblia	Gastric washings	McGrew (in Koss)	1968
L tropica	Skin	Lagerholm et al	1968
B coli	Peritoneal exudate	Lahiri et al	1977
Vorticella	Cervical	Hermann & Deininger	1963
T gondii	Vagina & cervix	San Cristobal & Roset	1976

Table IV *Trematodes Reported in Papanicolaou Smears*

Organism	Source	Authors	Year
P kellicotti ova	Aspirate of pulmonary cyst	McCallum	1975
Schistosoma spp ova	Vagina & cervix	Youssef et al	1962
	Vagina & cervix	Boulle & Notelovitz	1964
	Vagina & cervix	Berry	1966
	Vagina & cervix	Sharma et al	1970
	Vagina & cervix	Shennan & Gelfand	1971
	Vagina & cervix	Berry	1971
	Vagina & cervix	Berry	1976

Table V *Hydatid Sand Reported in Papanicolaou Smears*

Source	Authors	Year
Sputum	Sapunar & Klapp	1966
Aspirate of cyst of breast	Epstein	1969
Sputum	Allen et al	1972
Pleural effusion	Jacobson	1973
Cul-de-sac	Lahiri & Mitra	1976
Aspirate of cyst of liver	Garret et al	1977

Table VI *Nematodes Reported in Papanicolaou Smears*

Organism	Source	Authors	Year
T trichiura ovum	Vagina	*Zamorano et al*	1978
E vermicularis	Vagina	Langlinais[4]	1969
	Cervix	Rad & Jeannot	1970
	Vagina	Torres & Benitez Bribiesca	1973
	Vagina	San Cristobal & Mundt	1976
S stercoralis	Sputum	Kenny & Webber	1974
	Sputum	Patten	1976
	Sputum	Koss	1978
	Gastric & duodenal aspirate	Peralta & Rodrigues	1979
	Duodenal aspirate	Yassin & Garret	1980
W bancrofti microfilaria	Hydrocele fluid	Vassilakos & C	1974
	Vagina	Chandra et al	1975
	Nipple secretion	Lahiri	1975
M ozzardi microfilaria	Ascitic fluid	Figueroa	1973
D perstans microfilaria	Vagina	Jespers	1960
	Cervix	Sharma et al[44]	1971
O volvulus microfilaria	Cervix	Burges	1971

The parasites likely to be found in Papanicolaou smears are protozoa and larval stages of trematodes, cestodes and nematodes. Except for female *Enterobius* and *Strongyloides*, adult parasites are not likely to be found. The method stains parasites recognizably, though the appearances are unfamiliar to most parasitologists. The variety of body fluids, secretions and excretions submitted for cytopathologic examination are definitely more numerous than the material submitted for parasitologic examination. Thus, the chances are often greater that the cytopathologist will encounter parasites in normal or aberrant sources before the parasitologist will. The method lacks the possibility of studying the motility of parasites available in fresh smears. However, it certainly constitutes an extension of the tools for parasite diagnosis.

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References

1 Allen AR, Fullmer CD: Primary diagnosis of pulmonary echinococcosis by the cytologic technique. Acta Cytol 16 [illegible] 16 [illegible]

Berry A: A cytopathological and histopathological study of bilharziasis of the female genital tract. J Path Bact 91 325-338 1966

3 Berry A: Evidence of gynecologic bilharziasis in cytologic material: A morphologic study for cytologists in particular. Acta Cytol 15 [illegible] 197[illegible]

4 Berry A: Multispecies schistosomal infections of the female genital tract detected in cytology smears. Acta Cytol [illegible] 365 19[illegible]

5 Boyes K, [illegible] Findings of [illegible] larval stages in gynecologic smear. Acta Cytol 15 4 [illegible] 197[illegible]

6 Boulle [illegible] Bilharziasis of the female genital tract. S Afr [illegible] 1964

Chang [illegible] An unusual finding in the vaginal smear. Acta Cytol [illegible] 197[illegible]

8 Clark AH, Macker [illegible] Identification of trophozoites [illegible] by cytologic techniques. Acta Cytol [illegible] 197[illegible]

9 [illegible] Hydatid cyst of the breast: Diagnosis using cytologic techniques. Acta Cytol [illegible] 1964

1 [illegible] Papanicolaou [illegible] Edition [illegible] Lea & Febiger 19[illegible] pp [illegible]

11 F[illegible] [illegible] Acta Cytol [illegible] 168 169 197[illegible]

1 Figueroa JM: Presence of microfilariae [illegible] in ascitic fluid. Acta Cytol 17 73 75 19 3

13 Gartel M, Herbsman H, Ferst S: Cytologic diagnosis of echinococcosis. Acta Cytol [illegible] 553 554 1977

14 Hermann C, Denninger JT: Vorticella, an unusual protozoan found in endocervical smear. Acta Cytol 17 10 9 1030 1963

15 Hiller AS, Robel LR: Free living amebae identified by cytologic examination of gastrointestinal washings. Acta Cytol 18 59-61 19 4

16 Jacobson ES: A case of secondary echinococcosis diagnosed by cytologic examination of pleural fluid and needle biopsy of pleura. Acta Cytol 17 76 79 1973

17 Jespers J: Presence of microfilariae [illegible] in the vaginal smears of Congolese women. Gynecol Obstet 59 257 60 1960

18 Kenney M, Webber CA: Diagnosis of strongyloidiasis in Papanicolaou stained sputum smears. Acta Cytol 18 [illegible] 1974

19 Koss LC: Autoinfection with *Strongyloides stercoralis* (larva in sputum). Adventures in cytology. Meeting of New York Pathological Society, March 30 19 8

0 Koss LC: Diagnostic Cytology and Its Histopathologic Bases. Second edition. Philadelphia, JB Lippincott, 1968, pp 157 159 163 166 411-413 430 466 491

21 Lagerholm B, Gip L, Lodin A: The histopathological and cytological diagnosis of cutaneous leishmaniasis in two cases [illegible] Acta Derm Venere 148 600-607 1968

Lahiri VL: Microfilariae in nipple secretion. Acta Cytol 19 154 19 5

3 Lahiri VL, Elhence BI, Agarwal BM: Balantidium peritonitis diagnosed on cytologic material. Acta Cytol [illegible] 197[illegible]

4 Lahiri VL, Mitra R: Pelvic hydatid diagnosed from cytological material. Indian J Path Microbiol 19 63-65 1976

5 Langdon [illegible] *Enterobius vermicularis* in a vaginal smear. Acta Cytol 13 40-41 1969

6 Lefer LC, Rosier RI: Actinia in Pap smear. Acta Cytol [illegible] 45 197[illegible]

7 McCallum SM: Ova of the lung fluke *Paragonimus* [illegible] in fluid from a cyst. Acta Cytol 19 [illegible] 197[illegible]

8 McNeill KE, de Moraes Ruehsen M: Ameba trophozoites in cervicovaginal smear of a patient using an intrauterine device: A case report. Acta Cytol [illegible] 197[illegible]

9 Munguia H, Franco E, Valenzuela F: Diagnosis of genital amebiasis in women by the standard Papanicolaou technique. Am J Obstet Gynec 104 191 193 1969

30 Neefe JJ, [illegible] O, Gargani CF, Bauer H: Disseminated strongyloidiasis with cerebral involvement: A complication of corticosteroid therapy. Am J Med [illegible] 1973

31 Pai SK, [illegible] Lancet 1 5[illegible] 197[illegible]

[illegible] Diagnosis [illegible] Acta Cytol [illegible] 197[illegible]

33 [illegible] Acta Cytol [illegible] 197[illegible]

34 [illegible] Acta C[illegible]

Cytologic Findings

The smear stained by the Papanicolaou method was moderately cellular and contained mature squamous cells and many leukocytes The karyopyknotic index was approximately 50% and the superficial cells showed little folding or clumping An unexpected feature was the presence of scattered clumps of small epithelial cells containing dark-staining uniform nuclei and a small amount of cyanophilic cytoplasm (Figure 2) *There was polymorphonuclear infiltration* of many of the groups On closer inspection the nuclei which were dark blue appeared round or oval when *well* preserved they varied in size from approximately 7µm to 10µm The chromatin pattern showed a moderately coarse granularity and was distinguished by the presence of one two three or occasionally more large and rather irregular nucleoli (Figure 3) The nuclei appeared clearly defined by a smooth fine nuclear membrane though in slightly degenerated cells there occasionally was margination of some of the chromatin giving the inside of the membrane a beaded appearance

The surrounding cytoplasm stained a fairly deep green *though in some of the large clumps there was* retention of eosinophilic stain in the central parts Under high power the cytoplasm was seen to be stained unevenly, *and the cell borders were frequently* indistinct On the edge of the clumps and in single cells the cytoplasm often appeared drawn out and wispy (Figure 4)

Of particular interest one group of cells was found with three ciliated cells There was fairly close even spacing of the nuclei in the clumps of cells The cytoplasm on the edge of the clumps varied from a thin rim to a definite columnar border often there were rather wispy cytoplasmic projections While *there* was some variation in nuclear size granularity of the chromatin and amount of cytoplasm the overall impression was of an orderly arrangement of comparatively *uniform and distinctive* small columnar cells

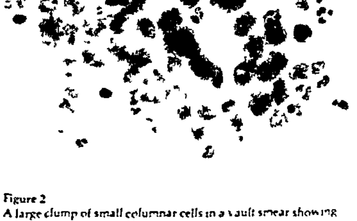

Figure 2
A large clump of small columnar cells in a vault smear showing polymorphonuclear infiltration and rather wispy cytoplasm (Papanicolaou stain × 40)

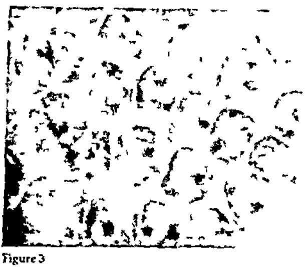

Figure 3
Oil immersion view of the columnar cells to illustrate the granular chromatin pattern and the large often multiple nucleoli in the otherwise regular and evenly spaced nuclei (Papanicolaou stain × 100)

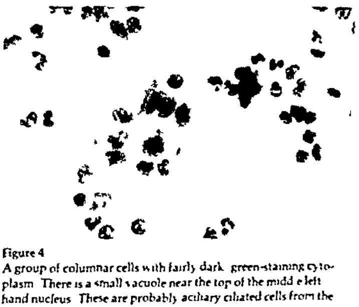

Figure 4
A group of columnar cells with fairly dark green-staining cytoplasm There is a small vacuole near the top of the middle left hand nucleus These are probably accessory ciliated cells from the fallopian tube (Papanicolaou stain × 40)

Discussion

It is very rare to find columnar cells in a vault smear and usually they are of malignant origin Frable[3] and his colleagues reviewed 2 206 slides that they had received labeled vaginal cuff and found that in 12 of the 39 slides that had significant cytologic atypia requiring some follow up the cervix was in fact still present in addition there was one case of vaginal endometriosis The small size of our cells and the rather coarse granular chromatin often with several irregular nucleoli did not correspond in appearance to either normal endocervical cells or typical clumps of endometrial cells A mesonephric duct is lined with low columnar epithelium which is nonciliated and nonsecretory and has a tendency to show squamous metaplasia [7] While the degenerate nature of the cytoplasm of the columnar cells in the vault made it difficult to ascertain the likely height of the columnar cells the later finding of a group of ciliated columnar cells excluded this diagnosis Vaginal adenosis another rare condition that could produce benign columnar cells in a vault smear can also be excluded since in this lesion the columnar cells have abundant cytoplasm and appear typical of normal endocervical type glandular epithelium

When the true diagnosis became apparent a search of the literature revealed detailed studies by Dudkiewicz[8,9] of the cytology of fallopian tube epithelial cells aspirated from 80 tubes removed at operation While four main types of epithelial cells are normally described on histologic examination of the tube[9,10]—namely the ciliated cell the secretory cell the peg or intercalary cell and an indifferent cell—Dudkiewicz also described three further cell types the cylindrical aciliary cell cells with protoplasmic junctions and cells with light -staining cytoplasm He considered that the first was probably a ciliated cell that had lost its cilia and that the other two were types of secretory cell the cells with protoplasmic junctions occurred at around ovulation and the light-staining cells were seen only after ovulation While the fallopian epithelial cells do not show such dramatic changes as those of the endometrium it is generally accepted that cyclical changes do occur in the tube

Since hysterectomy had been performed there was no precise information to indicate at which stage of the cycle the vault smear was taken From the cell pattern it seemed possible that the hormone levels were those found around midcycle or just after ovulation While it was hard to distinguish with certainty ciliary or cylindrical aciliary cells from secretory type cells apart from the few ciliated cells the overall appearances of the cells fitted in well with Dudkiewicz s description of cells at this stage of the cycle He stated that at midcycle the basophilic nuclei are oval or round and 8μm to 10μm in diameter with prominent and often multiple nucleoli and granular chromatin He found the secretory cells to be slightly larger columnar cells than the ciliated cells and their cytoplasm to be rather foamy as compared with the distinctively green cytoplasm of the ciliated cells which frequently had vacuoles over and under the nuclei That few of the cells in this case were ciliated could be due in part to the inflammatory changes as indicated by the leukocyte infiltration facilitating the loss of cilia while some of the slightly larger cells appeared similar to the secretory cells with protoplasmic junctions described by Dudkiewicz as occurring at midcycle (Figure 5)

The report by Carmichael[1] in 1976 of prolapse of the fallopian tube into the vaginal vault purported to be the 61st case to be described Most of these cases have followed vaginal hysterectomy [2-4,11] but Sumathy and Baucom[12] described three cases following abdominal hysterectomy In all three prolapse of the tube was preceded by pelvic infection In this patient prolapse appears to have followed spontaneous drainage of a vault hematoma One of the most common symptoms as in this case is a watery vaginal discharge but other symptoms that have been described include lower abdominal discomfort dyspareunia vaginal bleeding and pneumoperitoneum following coitus [2-4,11] Usually the prolapsed tube is mistaken for granulation tissue and the true diagnosis only comes to light following the failure of repeated cauterization of the tissue to cure the condition

Conclusion

We agree with Dudkiewicz s conclusion that if the

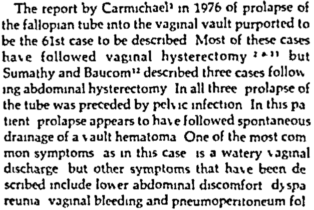

Figure 5

Slightly larger columnar cells with foamy cytoplasm similar to the secretory cells from the fallopian tube at midcycle as described by Dudkiewicz (Papanicolaou stain X 400) individual structure of the cells from the fallopian tube is kept in mind it should not be difficult to differentiate them from cells of endometrial or endocervical origin if they appear in the vaginal smear Since it is likely that prolapse of the fallopian tube is more common than the comparatively small number of reports in the literature suggest it is hoped that the description of this case may help others to make an early cytologic diagnosis of this lesion should it occur following either vaginal or abdominal hysterectomy thus saving some patients from repeated unavailing cauterization of vault granulations

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References

1 Burne JC Fallopian tube and pelvic inflammation *In* Post Graduate Obstetrical and Gynaecological Pathology Edited by H Fox FA Langley Oxford Pergamon Press 1973 pp 259 279

2 Carmichael DE Prolapse of the fallopian tube into the vaginal vault Am J Obstet Gynecol 125 266-267 1976

3 Dudkiewicz J Cytomorphology of epithelial cells of fallopian tubes obtained by aspiration from operative specimens Am J Obstet Gynecol 102 82-90 1968

4 Dudkiewicz J Quantitative and qualitative changes of epithelial cells of fallopian tubes in women according to the phase of menstrual cycle Acta Cytol 14 531 537 1970

5 Frable WJ Smith JH Perkins J Foley C Vaginal cuff cytology Some difficult diagnostic problems Acta Cytol 17 135-140 1973

6 Hart WR Zaharov I Kaplan BJ Townsend DJ Aldrich JO *Henderson BE Roy M Benton B Cytologic findings in stilbes*trol exposed females with emphasis on detection of vaginal adenosis Acta Cytol 20 7 14 1976

7 Langley FA Fox H Postgraduate Obstetrical and Gynaecological Pathology Oxford Pergamon Press 1973 p 83

8 Lehto L Cytology of the human fallopian tube Acta Obstet Gynecol Scand 42 1 77 1963

9 Novak ER Woodruff JD Gynecologic and Obstetric Pathology Seventh edition Philadelphia WB Saunders 1974 pp 288 312

10 Pauerstein CJ The Fallopian Tube A Reappraisal Philadelphia Lea & Febiger 1974 pp 21 22

11 Pitkin RM Scott JR The Year Book of Obstetrics and Gynecology Chicago Year Book Medical Publishers 1977 pp 258-259

12 Sumathy V Baucom K Prolapse of the fallopian tube following abdominal hysterectomy Int J Gynaecol Obstet 13 273-276 1975

Psammoma Bodies and Detached Ciliary Tufts in a Cervicovaginal Smear Associated with Benign Ovarian Cystadenofibroma

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Psammoma bodies and detached ciliary tufts or ciliocytophthoria were present in a cervicovaginal smear from a 38 year-old woman with a benign cystadenofibroma of the left ovary This case demonstrates that psammoma bodies in cervicovaginal smears may occur in association with nonmalignant conditions of the female genital tract and that detached ciliary tufts may originate from the ovarian epithelium

Psammoma bodies are rarely found in cervicovaginal cell preparations When present they are regarded as presumptive evidence of papillary carcinoma of the ovary[1,2,7,12] or a less frequent type of endometrial carcinoma with psammoma bodies[14] Psammoma bodies however may originate from benign conditions of the female genital tract including benign proliferative ovarian lesions and reparative lesions involving surface epithelium of the ovary and fallopian tubes and are rarely associated with benign endometrial pathology[5,8-11]

Detached ciliary tufts (DCTs) or ciliocytophthoria a common finding in sputum in viral infections are rarely encountered in gynecologic smears They have been described in ovarian cyst fluid and occasionally in cervicovaginal smears[6,10,13] These DCTs are thought to result from cellular degeneration of ciliated epithelium from the cervix endometrium fallopian tube or ovary[13]

This case demonstrates the unusual finding of psammoma bodies along with DCTs in a cervicovaginal smear associated with benign cystadenofibroma of the ovary

Case Report

K M a 38 year-old gravida 2 para 1 obese female presented complaining of intermenstrual bleeding and prolonged irregular menstrual periods for almost ten years Menarche was at age 15 and her last menses were 23 days prior to the current visit She had a right salpingo-oophorectomy for a benign cyst of the ovary 15 years before

The physical examination revealed normal external genitalia cervix and uterus There was a large cystic movable nontender left adnexal mass A cervicovaginal smear showed papillary clusters and psammoma bodies suggestive of malignancy of probable ovarian origin

Sonography revealed a 6×6×5-cm cystic mass in the left ovary The uterus was of normal size

At exploratory laparotomy a 10-cm cystic left ovarian mass and a normal fallopian tube were seen A total abdominal hysterectomy with left salpingo-oophorectomy was carried out

The postoperative course was uneventful The patient is symptom free three years postoperatively

Cytologic Findings

The cervicovaginal smear showed multiple papillary clusters of various sizes some containing single or multiple psammoma bodies (Figure 1) Individual cells exhibited a slight variation in size and shape and

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metrial lesions in women on oral contraceptives[14] with questionable tuberculous endometritis[7] or with intrauterine devices[5]

In our case the psammoma bodies originated in a cystadenofibroma of the left ovary The tumor is usually a benign neoplasm with proliferation of the surface epithelium of the ovary and a prominent fibrous tissue component derived from the ovarian cortex Calcification and psammoma bodies are present in about 30% of cystadenofibromas of the ovary[3]

DCTs have been described in cervicovaginal smears however their significance remains unclear The true incidence of their occurrence is not known and is difficult to assess because they may easily be overlooked DCTs are essentially fragments of degenerating ciliated cells from different sources including the endocervix and endometrium[6,10,13] they have also been seen in ovarian cyst fluid[9] In the current case DCTs in the smear were present in the vicinity of cells of ovarian neoplastic origin thus indicating their possible origin in the ovary

References

1 Benson PA Psammoma bodies found in cervicovaginal smear Case report Acta Cytol 17 64-66 1973

2 Beyer Boon ME Psammoma bodies in cervicovaginal smear An indicator of the presence of ovarian carcinoma Acta Cytol 18 41-44 1974

3 Czernobilsky B Borenstein R Lancet M Cystadenofibroma of the ovary Cancer 34 1971 1981 1974

4 Ferenczy A Talens M Zoghby M Hussan SS Ultrastructural studies on the morphogenesis of psammoma bodies in ovarian serous neoplasia Cancer 39 2451 2459 1977

5 Highman WJ Calcified bodies and the intrauterine device Acta Cytol 15 473-475 1971

6 Hollander DH Gupta PK Detached ciliary tufts in cervicovaginal smears Acta Cytol 18 367 369 1974

7 Jenkins DM Goulden R Psammoma bodies in cervical cytology smears Acta Cytol 21 112 113 1977

8 Koss LG Diagnostic Cytology and Its Histopathology Bases Third edition Philadelphia JB Lippincott 1979 pp 481-482

9 Marinaccio G Pollice L Risso G Bete F Unusual ciliated epithelial cells from an ovarian cyst Acta Cytol 18 65-67 1974

10 Muller Kobold Walterbeck AC Beyer Boon ME Ciliocytophthoria in cervical cytology Acta Cytol 19 89-91 1975

11 Picoff RC Meeker C Psammoma bodies in the cervicovaginal smear in association with benign papillary structures of the ovary Acta Cytol 14 45-47 1970

12 Qizilbash AH Ovarian carcinoma identified by psammoma bodies in vaginal smear Can Med Assoc J 110 185-186 1974

13 Rilke F Alasio L Detached ciliary tufts in cervicovaginal smear Acta Cytol 20 189 190 1976

14 Valicenti JF Priester SK Psammoma bodies of benign endometrial origin in cervicovaginal cytology Acta Cytol 21 550-552 1977

Pleural Effusion in Systemic Lupus Erythematosus

Mohammad G Reda M D
Walter Baigelman M D

Three cases of systemic lupus erythematosus (SLE) with pleural effusion are reviewed The characteristics of the effusions are presented and the literature pertaining to lupus related effusions is reviewed There is great heterogeneity in lupus pleural effusions The presence of polymorphonuclear neutrophils as the predominant white cell while consistent with SLE should raise the possibility of complicating bacterial infection The presence of lupus erythematosus (LE) cells in the fluid seems to be the most specific diagnostic criterion

Pleural involvement is a common feature of systemic lupus erythematosus (SLE)[illegible,10] with pleuritic chest pain a symptom at some time during the course of the disease in 45% to 56% of patients[illegible,10] However pleural effusion is less common Harvey et al reported pleural effusion in only 16% of their patients[10] and Dubois in 30 3% of his patients[illegible]

Although pleural effusion is usually a late manifestation in a known case of SLE it may be among the earliest manifestations of the disease[13,14] When pleural disease is a presenting feature examination of pleural fluid might aid in establishing a correct diagnosis Unfortunately little has been written about the characteristics of pleural effusions in patients with SLE

We have encountered three cases of SLE with pleural effusion Several interesting observations were made that may help one to better understand this aspect of the disease

Case Reports

Case 1

A 64 year-old man was admitted to the hospital with a two month history of bilateral pleuritic chest pain associated with increasing fatigue weakness and anorexia Eighteen months before admission he developed arthritis involving the small joints of both hands

Physical examination revealed normal vital signs and a respiratory rate of 18 per minute Skin and mucous membranes were normal Breath sounds were decreased at both bases and no friction rub was auscultated The limbs were not edematous or deformed but soft tissue swelling of the proximal interphalangeal joints of the hands the metacarpophalangeal joints and the knees was noted Poor grip in both hands and limitation of the range of motion of both wrists were present

Chest roentgenogram demonstrated bilateral small pleural effusions Laboratory data revealed a white blood count of 9 700 per cu mm with 81% neutrophils 3% bands 10% lymphocytes and 6% monocytes The hematocrit was 34% and the smear of the peripheral blood showed microcytic cells The erythrocyte sedimentation rate (ESR) was 119 ml/hour (*Westgreen*) creatinine 1 3 mg/dl and fasting blood glucose 160 mg/dl

Thoracentesis was performed on the second hospital day Twelve milliliters of serosanguinous fluid was obtained from the left pleural cavity Chemical examination of the fluid showed a protein of 4 8

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gm/dl lactic dehydrogenase of 590 mU/ml and glucose of 110 mg/dl There were 120 000 red blood cells per milliliter and 20 000 white blood cells per milliliter with a differential count of 96% neutrophils 1% lymphocytes and 3% plasmalike cells One cell suspected of being a lupus erythematosus (LE) cell was seen on the smear stained with Wright s stain On the following day the thoracentesis was repeated Fifty milliliters of fluid with characteristics similar to those of the first aspirate was sent to the laboratory for staining with Wright s stain Inadvertently the fluid was not stained until about 18 hours later The smear then demonstrated numerous typical LE cells (Figure 1) LE cells were also found in the peripheral blood

The serum antinuclear antibodies (ANA) were positive in a speckled pattern with a titer of 1 8 192 antideoxyribonucleic acid (anti DNA) antibodies were also positive The patient responded well to therapy including aspirin and hydroxychloroquine

Case 2

A 47-year-old woman was admitted with complaints of fever and malaise The diagnosis of SLE had been made two months earlier with positive tests for antinuclear antibodies and anti DNA antibodies Two weeks before admission she was found to have a urinary tract infection which was treated with ampicillin Recurrence of fever prompted this admission

Vital signs on admission were a temperature of 38 9 C with tachypnea and tachycardia Skin and mucous membranes were normal There was dullness to percussion and decreased breath sounds at both lung bases and a cardiac friction rub was heard Her hematocrit was 27% and her white blood cell count was 12 000 per cu mm with a normal differential The ESR was 95 mm/hour and creatinine 1 mg/dl Urinalysis revealed numerous white and red blood cells Urine cultures were negative

Chest roentgenogram showed an enlargement of the cardiac silhouette and bilateral pleural effusions Echocardiogram confirmed the presence of a pericardial effusion Antinuclear antibodies were positive at a titer of 1 1 024 The anti DNA antibody titers were also elevated

Thoracentesis performed on admission yielded straw-colored fluid with a protein of 3 2 gm/dl (serum protein 6 8 gm/dl) glucose of 115 mg/dl and lactic dehydrogenase of 210 mU/ml Microscopic examination revealed 8 000 red cells per milliliter and 15 900 white blood cells per milliliter with a differential count of 80% neutrophils 2% lymphocytes and 10% mesothelial cells LE cells were not seen Gram stain and cultures of the fluid were negative Pleural fluid complement levels were C3 less than 62 4 mg/dl and C4 less than 10 mg/dl Gentamicin therapy was initiated for suspected urinary tract infection After several days of no clinical response and with all the cultures negative therapy with antibiotics was discontinued Daily treatment with 60 mg

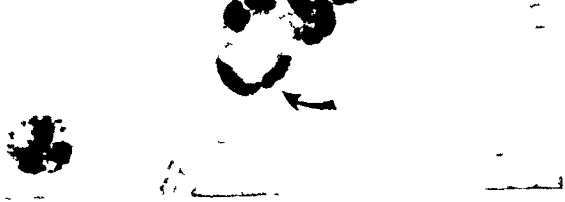

Figure 1
Pleural fluid Lupus erythematosus cells (Wright s stain × 100)

of prednisone was begun and there was a rapid clinical response including resolution of the fever and the pleural and pericardial effusions

Case 3

A 60-year-old woman was admitted with a four-day history of fever decreased appetite nausea and cough A diagnosis of SLE had been made about one year earlier on the basis of fever arthritis mental confusion an elevated ESR leukopenia and anemia with a normal bone marrow Additional supporting laboratory data at the time of initial diagnosis included positive antinuclear antibodies in a homogeneous pattern with a titer of 1 33 000 and a total serum complement of 75 mg/dl (normal 120 to 170) Coombs s tests anti DNA antibodies and LE preparations were negative

Physical examination revealed the patient to be in mild respiratory distress A low-grade temperature was noted and an irregular pulse rate of 150 per minute was recorded Chest examination revealed dullness to percussion at the right base with decreased breath sounds and bibasilar rales A chest roentgenogram demonstrated cardiomegaly a moderate-sized right-sided pleural effusion and some atelectasis in the lower third of the left lung The white blood cell count was 7 500 per cu mm with 77% neutrophils 17% bands and 5% lymphocytes The hematocrit was 33% and the ESR was 61

Thoracentesis performed on admission obtained 100 ml of serosanguinous fluid with a protein content of 3 8 gm/dl (serum protein 6 4) lactic dehydrogenase content of 3 080 mU/ml and glucose content of 17 mg/dl There were 2 600 white blood cells per milliliter composed of 71% neutrophils 23% lymphocytes and 6% mesothelial cells A gram stain was not performed Cultures of the pleural effusion blood and sputum grew *Streptococcus pneumoniae* After a complicated hospital course the patient was discharged

Discussion

Infection uremia and congestive heart failure are some of the complications of SLE that are capable of causing pleural effusions Therefore it is essential that the cause of the effusion be ascertained in any patient suspected of having or known to have SLE Review of the literature is of little assistance in characterizing the unique features of pleural effusions caused by SLE A major textbook on autoimmune diseases ignores the issue [18] and a recent textbook on pulmonary diseases states The available data are inadequate to differentiate between lupus and other causes of effusion on the basis of cellular details or types of effusion [9] Large series of cases of SLE[5,7,8,10] emphasized the occurrence of pleural effusions without describing the fluid Winslow [19] in an article emphasizing the importance of pleuritis and pleural effusion in the diagnosis of SLE failed to give any description of the fluid

Part of the difficulty of describing the pleural effusions may have been the absence of acceptable criteria for what should be regarded as distinguishing characteristics Light et al[15] seemed to have reached acceptable criteria for distinguishing between transudates and exudates Most recent papers that contain the description of pleural effusions in SLE emphasize only one characteristic and fail to give a full description (Table I) Although Dubois considered the pleural effusion of SLE to be a *transudate* most of the

Table I [illegible]

	No of patients	No of exudates	White cells	Comment
Katz 1958	1	NR	NR	LE cell seen
[illegible] 1970	10	0	NR	LE cell not seen
Kelley 1971	10	10	Lymphocytes histiocytes plasma cells and neutrophils in varying proportion	LE cell not seen
Dutta 197[illegible]	1	1	NR	Granuloma on pleural biopsy
[illegible] 197[illegible]	6	31	NR	Low fluid complement
Land[illegible] 1976	1	1	WBC [illegible]	LE cell seen on hematoxylin and eosin stain
Ca[illegible] 19[illegible]	1	1	WBC [illegible]	LE cell seen on Wright's stain

[illegible] reported
[illegible] LDH [illegible]
[illegible] LDH [illegible] reported

Cell Content in Repetitive Canine Bronchoalveolar Lavage

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To determine the effects and effectiveness of small volume (50 ml) bronchoalveolar lavage lavages were performed on healthy mongrel dogs under a number of protocols The differential and total cell counts in small volume bronchoalveolar lavages were determined These factors were compared to those obtained by large volume (500 to 600 ml) lavage of the same segment in a second experimental group Small volume lavage yielded a greater total cell count per milliliter and a greater concentration of polymorphonuclear leukocytes (PMNs) than large volume lavage in which there was a somewhat higher co centration of bronchial cells We compared the ce populations from lobes lavaged at 48 hour interval No significant changes in these populations or in pu monary histology were noted

Our results suggest that small volume bronchoa veolar lavage provides a representative sample of th total free cell yield from the lower respiratory trac Furthermore small volume bronchoalveolar lavag performed at 48 hour intervals does not influence th distribution of these cells or significantly damage th pulmonary parenchyma

Bronchoalveolar lavage has been used to characteriz cell populations in noncellular elements in norma and diseased human lungs and various models of pulmonary and systemic disease [1,2,4,6,8,9] Large volum bronchoalveolar lavage however has been reporte to be associated with a significant morbidity in certain canine models [3] In this study we sought to describe the normal cellular content that can be expected from a single small volume lavage and from repetitive small volume lavage in healthy mongre dogs We also sought to evaluate the effects of bronchoalveolar lavage on pulmonary parenchymal histology

Materials and Methods

Healthy mongrel dogs weighing between 15 and 25 kg with normal chest roentgenograms and without cough or rhinorrhea were lightly anesthetized with intravenous pentobarbitol placed in the supine position with the lower lobes slightly dependent and intubated with an endotracheal tube These animals underwent bronchoalveolar lavage through the suction irrigation channel of a fiber optic bronchoscope (Machida model FBS-6T Machida America Norwood New Jersey) wedged into a subsegmental

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bronchus of about 4 mm in diameter The animals were studied utilizing four protocols

Group 1 Small Volume Lavage

Twenty five animals underwent a lavage of 75 separate lobes with a single bolus of 50 ml of 0 9% sodium chloride instilled at 25 C After 10 to 20 seconds the solution was aspirated nontraumatically and collected in a sterile Lukens trap After thorough mixing a 2 ml sample was taken to determine total cell count in a hemocytometer the remainder which constituted 50% to 60% of the instilled aliquot was immediately fixed in 50% ethyl alcohol and prepared as described below for determination of differential cell counts Representative animals from this group underwent multiple open lung biopsies of the appropriate segments within two hours after the lavage was completed

Group 2 Small Volume Lavage versus Large Volume Lavage

Six animals underwent lavage of a single subsegmental bronchus with a 50 ml bolus of 0 9% sodium chloride as described above Immediately upon completion of this lavage these animals had a 100 ml loading volume of 0 9% sodium chloride instilled in a previously lavaged segment They then underwent five 100-ml lavages of that area The lavagate was aspirated after each instillation and the total return *about 80%* was pooled A sample was removed for measurement of total cell count and the remainder was fixed *in* 50% alcohol and prepared for determination of differential cell counts

Group 3 Multiple Aliquot Lavages

Five animals underwent ten serial 50-ml lavages with 0 9% sodium chloride in two widely separated subsegmental bronchi The fluid from each lavage was collected separately a sample was removed for the measurement of total cell count and the remainder was fixed in 50% alcohol and prepared for the determination of differential cell counts

Group 4 Multiple Alveolar Lavages

Seven animals underwent small volume saline lavage of a specific subsegmental bronchus at 48 hour intervals The right upper lobe the right lower lobe the left upper lobe and the left lower lobe were lavaged *in* each animal in a staggered manner so that at the time of completion of the study from one to four lavages had been performed in each lobe After the fourth lavage was completed the animals were killed and biopsies were taken from each of the lavaged lobes Total and differential cell counts were performed on the individual lavage specimens

Group 5 Regional Cell Counts

Five animals underwent small volume saline lavage in subsegments of each of the major lobes Total and differential cell counts were performed on each lavage specimen

Methodology for Differential Cell Counts

Lavaged specimens were centrifuged at 750 rpm for 15 minutes The supernatant was decanted and five smears were prepared on albuminized slides from the remaining cell plug The slides were immediately fixed in 95% ethyl alcohol and stained by the Papanicolaou method Differential cell counts were performed by classifying a minimum of 200 cells on each slide Polymorphonuclear leukocytes (PMNs) pulmonary macrophages lymphocytes small mononuclear cells and bronchial epithelial cells were identified by their morphologic features Total cell counts were estimated by standard methods in a counting chamber

Histologic Evaluation

Within two hours of completing the lavage study specimens for microscopic examination were obtained from the lungs of animals in groups 1 *and* 4 Multiple sections were taken from previously lavaged areas fixed in 10% formalin embedded in paraffin and stained with hematoxylin and eosin for light microscopic evaluation Serial sections were obtained from the lungs of animals in which high lavage PMN counts (above 10%) suggested the presence of pulmonary parenchymal infection

Results

Cytologic data from groups 1 through 4 are summarized in Table I The data were subjected to statistical analysis with the two tailed Wilcoxon matched pair signed rank test[19]

Group 1 Small Volume Lavage

With a single 50 ml saline lavage the mean PMN count was 15% Despite a large observed range the percentage of PMNs obtained in 69 of 75 lavages fell within one standard deviation of the mean (*Figure 1*) Histologic examination of the lungs in this group of animals was essentially normal except fo scattered areas of interstitial cellular infiltrates and edema There was no evidence of acute pulmonary paren

Table I *Summary of Data Obtained under the Various Lavage Protocols (Percentage of Cells Obtained)*

	No of dogs	PMNs	Alveolar macrophages	Lymphs monos	Bronchial cells	Cell counts/ml	
						Range	Mean
1 Small volume lavage	25	15±9.3 (1-48)	54±10.5 (34-80)	11±8.8 (1-45)	18±11 (2-40)	0.01×10^{6}-5.0×10^{6}	0.37×10^{6}
2 Small volume lavage versus	6	18±7.9 (6-36)	56±4.7 (48-61)	11±4.9 (4-22)	15±7.7 (6-30)	0.22×10^{6}-1.2×10^{6}	0.72×10^{6}
large volume lavage		5.6±3.1 (2-12)	55±16.7 (12-66)	15±7.7 (7-28)	25±14 (17-57)	0.08×10^{6}-0.88×10^{6}	0.38×10^{6}
3 First lavage versus	5	26±13 (14-48)	55±15.6 (34-75)	7±3.4 (3-12)	13±7.3 (2-23)	0.12×10^{6}-1.1×10^{6}	0.48×10^{6}
tenth lavage		12±9 (1-28)	61±13 (42-81)	6±5.4 (2-20)	21±12.3 (6-45)	0.05×10^{6}-0.32×10^{6}	0.15×10^{6}
4 First lavage versus	7	11±4.8 (8-21)	56±13.6 (40-70)	15±15 (2-45)	16±12 (7-22)	0.16×10^{6}-1.1×10^{6}	0.56×10^{6}
fourth lavage (at 48 hour intervals)		12±7.1 (4-20)	50±7 (41-59)	18±12 (4-33)	18±10 (2-30)	0.2×10^{6}-4.3×10^{6}	0.9×10^{6}

Data are expressed as mean ± standard deviation with range in parentheses below

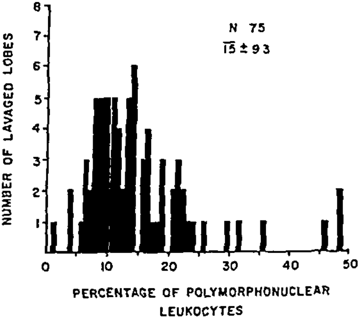

Figure 1
The distribution of polymorphonuclear leukocytes obtained by a single 50-ml saline lavage

chymal or bronchial infection in any of the animals studied

Group 2 Small Volume Lavage versus Large Volume Lavage

There was a greater concentration of PMNs in small volume lavage than in large volume lavage (Figure 2) The concentration of other cells obtained did not significantly differ using the two techniques with the *exception of the somewhat greater concentration of* bronchial cells in the large volume lavage These differences were significant at a P value of less than 0.01 In addition as shown in Table I mean cell counts were reduced by approximately half with large volume lavage

Group 3 Multiple Aliquot Lavage

Initial PMN percentages in this group of animals were reduced more than 50% by the tenth sequential lavage (Figure 3) These differences were significant at a P value of less than 0.01 A decreasing percentage of PMNs was observed with each lavage usually *declining to a plateau by the seventh or eighth aliquot* Mean cell counts consistently decreased by as much as a factor of ten in individual animals by the tenth lavage The initial aliquots accounted for the majority of cells obtained by the total lavage pool In addition the percentage of bronchial epithelial cells increased with each lavage the final lavages tended to yield sheets of these cells

Group 4 Multiple Alveolar Lavage

Comparison of *differential cell counts from* lobes lavaged one to four times at 48-hour intervals revealed no significant differences in the percentage of cells (Figure 4) Gross inspection of the lavaged lobes revealed an occasional small area of consolidation There was no correlation between the number of times the lobe was lavaged and the frequency with which these areas were observed Microscopic examinations of sections from the lavaged lobes revealed

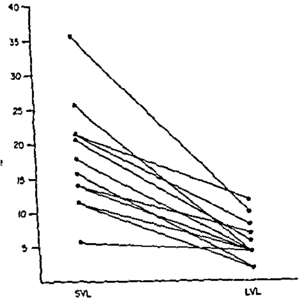

Figure 2
The percentage of polymorphonuclear leukocytes in small volume (SVL 50 cc) versus large volume (LVL 600 ml) canine alveolar lavage (P value < 0 01)

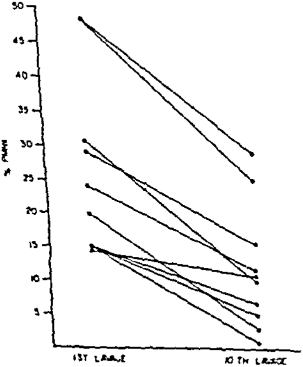

Figure 3
The effect of ten sequential 50 ml lavages upon the percentage of polymorphonuclear leukocytes (P value < 0 01)

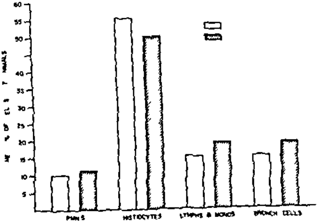

Figure 4
Differential cell counts in canine alveolar lavage first versus fourth lavage of the same subsegment at 48 hour interval

Table II Percentage of Polymorphonuclear Leukocytes in Small Volume Consecutive Lavage (Group 5) by Site

	Dog number				
	1	2	3	4	5
RUL	6	7	17	19	23
RLL	10	8	11	17	23
LUL	12	20	15	15	4
LLL	15	15	14	29	31
Mean	11±3 8	13±6 1	14±2 5	20±6 2	25±3 9

Mean for all group 5 dogs = 17±6 9
(Mean for all group 1 dogs = 15±9 3)

RUL = right upper lobe
RLL = right lower lobe
LUL = left upper lobe
LLL = left lower lobe

occasional focal areas of interstitial edema and intra alveolar hemorrhage These areas occurred as frequently in lobes undergoing a single lavage as in those undergoing multiple lavages

Group 5 Regional Cell Counts

The percentage of PMNs from lobes consecutively lavaged in the same animal are shown in Table II Mean PMN counts from each individual animal and from the group as a whole were equivalent to those in group 1 Individual animals tended to exhibit similar counts in each of their lavaged lobes (Table II)

Chest Roentgenograms

Chest roentgenograms taken within 24 hours after lavage revealed occasional infiltrates which in all cases resolved without clinical evidence of

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	No of dogs	PMNs	Alveolar macrophages	Lymphs monos	Bronchial cells	Cell counts ml	
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large volume lavage		5.6±3.1 (2–12)	55±16.7 (12–66)	15±7.7 (7–28)	25±14 (17–57)	0.06×10^6–0.88×10^6	0.36×10^6
3 First lavage versus tenth	5	26±13 (14–48)	55±15.6 (34–75)	7±3.4 (3–12)	13±7.3 ([illegible]–23)	0.12×10^6–1.1×10^6	0.45×10^6
lavage		12±9 (1–28)	61±13 (42–81)	6±5.4 (2–20)	21±12.3 (6–45)	0.0[illegible]×10^6–0.32×10^6	0.15×10^6
4 First lavage versus fourth	7	11±4.8 (8–21)	56±13.6 (40–70)	15±15 (2–45)	16±12 (7–2[illegible])	0.16×10^6–1.1×10^6	0.56×10^6
lavage (at 48-hour intervals)		*12±7.1* (4–20)	50±7 (41–59)	*18±12* (4–33)	*18±10* (2–30)	*0.2×10^6–4.3×10^6*	*0.9×10^6*

Data are expressed as mean ± standard deviation with range in parentheses below

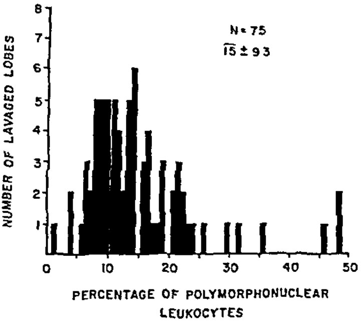

Figure 1
The distribution of polymorphonuclear leukocytes obtained by a single 50 ml saline lavage

chymal or bronchial infection in any of the animals studied

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Initial PMN percentages in this group of animals were reduced more than 50% by the tenth sequential lavage (Figure 3) These differences were significant at a P value of less than 0 01 A decreasing percentage of PMNs was observed with each lavage usually declining to a plateau by the seventh or eighth aliquot Mean cell counts consistently decreased by as much as a factor of ten in individual animals by the tenth lavage The initial aliquots accounted for the majority of cells obtained by the total lavage pool In addition the percentage of bronchial epithelial cells increased with each lavage the final lavages tended to yield sheets of these cells

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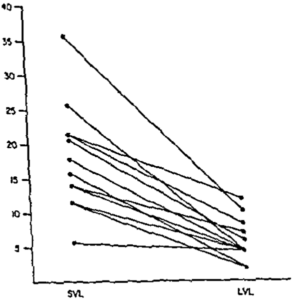

Figure 2
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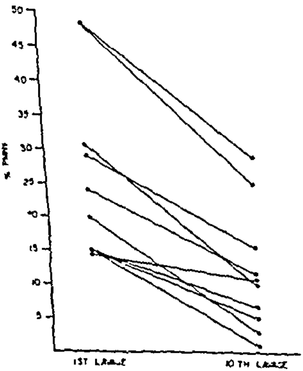

Figure 3
The effect of ten sequential 50 ml lavages upon the percentage of polymorphonuclear leukocytes (P value < 0.01)

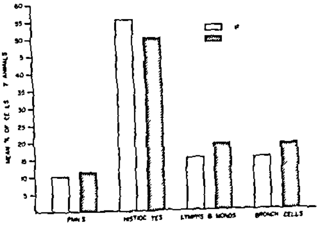

Figure 4
Differential cell counts in canine alveolar lavage first versus fourth lavage of the same subsegment at 48 hour interval

Table II Percentage of Polymorphonuclear Leukocytes in Small Volume Consecutive Lavage (Group 5) [illegible]

	Dog number				
	1	2	3	4	5
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LUL	12	20	15	15	24
LLL	15	15	14	29	31
Mean	11 ± 3.8	13 ± 6.1	14 ± 2.5	20 ± 6.2	25 ± 3.9

Mean for all group 5 dogs = 17 ± 6.9
(Mean for all group 1 dogs = 15 ± 9.3)

RUL = right upper lobe
RLL = right lower lobe
LUL = left upper lobe
LLL = left lower lobe

occasional focal areas of interstitial edema and intraalveolar hemorrhage. These areas occurred as frequently in lobes undergoing a single lavage as in those undergoing multiple lavages.

Group 5 Regional Cell Counts

The percentage of PMNs from lobes consecutively lavaged in the same animal are shown in Table II. Mean PMN counts from each individual animal and from the group as a whole were equivalent to those in group 1. Individual animals tended to exhibit similar counts in each of their lavaged lobes (Table II).

Chest Roentgenograms

Chest roentgenograms taken within 24 hours after lavage revealed occasional infiltrates which in all cases resolved without clinical evidence of pulmonary infection Animals undergoing multiple lavages of the same lobe showed no greater incidence of infiltrates than did those lavaged a single time

Discussion

On the basis of our roentgenographic and histologic studies we believe that the recovery of a high concentration of PMNs by bronchoalveolar lavage does not always reflect active pulmonary infection Prior studies utilizing large volume bronchoalveolar lavage in dogs have suggested that high cell counts and increased numbers of PMNs in the lavagate were caused by pulmonary disease [6] These investigations however were not validated by pathologic examination of the lavaged lungs The only abnormalities we encountered in the lungs of our experimental animals were focal collections of inflammatory cells and minimal interstitial edema which we feel were secondary to the lavage procedure itself Moreover these changes were found with no greater frequency in lungs that yielded high as opposed to low PMN counts

Our data demonstrate that the volume of the lavage fluid instilled may influence the total cell count and the concentration of the various cells recovered We have shown that lavage with several large aliquots or with multiple small aliquots yielded a lower concentration of PMNs and a higher concentration of bronchial cells than did a single 50 ml lavage Analysis of the cell content obtained when lungs were lavaged with multiple 50 ml aliquots demonstrated that the first several aliquots contained the majority of the total cells recovered as well as the highest concentration of PMNs The concentration of PMNs declined with each succeeding lavage aliquot and reached a plateau by the fifth or sixth instillation with approximately two thirds of the total number of PMNs recovered in the first 150 ml

These observations suggest that a single 50-ml lavage or the initial aliquot of serial 50 ml lavages removes the less adhesive or free cells from the bronchoalveolar tree Subsequent lavage aliquots contribute cells that are less easily removed from their basement membranes or from the peribronchial or interstitial spaces The higher concentration of bronchial lining cells obtained thus indicates that a large total volume may be more traumatic to the bronchial tree than a single small volume lavage

Our data confirm and extend the observation that repeated lavage of the same lobar segment at widely spaced intervals (48 hours) does not affect the concentration or the type of bronchoalveolar cells recovered or cause significant changes in pulmonary morphology Our study of lobes lavaged from one to four times at 48 hour intervals revealed no increase in PMNs over baseline levels At the time of the animals deaths after the fourth lavage evaluation of histologic sections of the lavaged lobes showed no pathology except for scattered areas of inflammatory cells and interstitial edema The frequency with which these areas occurred did not correlate with the frequency of lavage These observations support the safety of repetitive monitoring of bronchoalveolar cytology thus permitting the study in canine models of the evolution of pulmonary lesions of various human pulmonary diseases

While saline lavage in rhesus monkey lungs causes an increase in PMNs perhaps by release of chemotactic factors by activated macrophages [7] we did not observe a similar phenomenon in our serial lavages of canine lungs An equivalent mechanism may not be present in the canine lung or if present may follow a more rapid time course and therefore was not detected by the time of our repeat lavage

We found that the cell population of normal canine lungs is essentially uniform In comparing cell counts obtained at the same time in various lobes of the same animal we failed to find any regional differences in cell distribution It is possible that regional perfusion inequalities may influence the type of cells present in the various pulmonary segments but such differences were not observed in our preliminary studies

Our results suggest that small volume lavage provides a representative sample of the cells from the lower respiratory tract and that repetitive small volume lavage does not damage the pulmonary parenchyma or change the distribution of the cells recovered

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References

1 Achterrath U Blumcke S Koerner SK Yipintsoi T Siegelman SS Chandler P Hagstrom JWC Torres M Cobbah JE Fujii P Veith FJ Alveolar lavage cytology in transplanted lungs J Thorac Cardiovasc Surg 69 510 520 1975

2 Daniele RP Altose MD Salisbury BG Rowlands DT Characterization of lymphocyte populations in normal human lungs Chest 67 52S 53S 1975

3 Drews JA Shimada K White J H Benfield JR The pulmonary alveolar macrophage in lung transplantation Transplantation 17 319 3 1974

4 Finley TN Swenson EW Curran WS Luber GL Ladman AJ Bronchopulmonary lavage in normal subjects and patient with obstructive lung disease Ann Intern Med 66 651-658 1967

5 Kaltreider HB Salmon SE Immunology of the lower respiratory tract J Clin Invest 52 2211 2217 1973

6 Kaltreider HB Turner FN Salmon SE A canine model for comparative study of respiratory and systemic immunologic reactions Am Rev Respir Dis 111 257 265 1975

7 Kazmierowski JA Gallin JI Reynolds HY Mechanism for the inflammatory response in primate lungs J Clin Invest 59 273 281 1977

8 Pratt SA Finley TN Smith MH Ladman AJ A comparison of alveolar macrophages and pulmonary surfactant obtained from the lungs of human smokers and non-smokers by endobronchial lavage Anat Rec 163 497 507 1969

9 Reynolds HY Fulmer JD Kazmierowski JA Roberts WC Frank MM Crystal RS Analysis of cellular and protein content of broncho alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis J Clin Invest 59 165 175 1977

10 Sokal RR Rohlf FJ Biometry The Principles and Practice of Statistics in Biological Research San Francisco WH Freeman 1969 p 400

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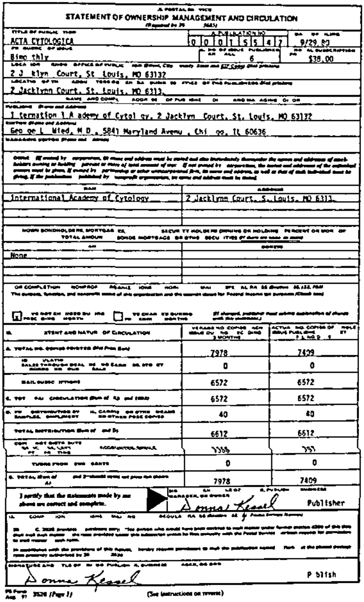

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	0	0
	6572	6572
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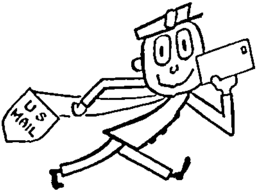

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